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The Journal of Laboratory and Clinical Medicine

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CLINICAL AND EXPERIMENTAL

A BACTERIOLOGIC STUDY OF FIVE HUNDRED SIXTY-SEVEN POSTMORTEM EXAMINATIONS*

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THE value of most postmortem examinations is enhanced tremendously if a careful bacteriologic study is made on the fluids and organs of the body; in many instances the entire solution as to the cause of the patient's illness and death can only be reached by such bacteriologic research.

In the presence of abscesses, peritonitis, pneumonia, etc., if a specific organism is found the diagnosis is complete. The finding of Actinomyces, Streptothrix and amebae has a similar significance. The work of the bacteriologist is even more necessary when for various reasons proper studies cannot be made before death. Hence the cause of death which may have been obscure can either be definitely determined or corroborated.

Judging from the literature postmortem bacteriology has received little attention and moreover is receiving even less in recent years. Many problems present themselves in this phase of bacteriology and certain workers in this field consider the results so conflicting as to be unsatisfactory. The questions confronting one in the interpretation of the results obtained in a study of this problem are: Is there a terminal or agonal invasion of the blood stream

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and tissues of the body by bacteria already present within the gastrointestinal and pulmonary tracts? Is this terminal invasion of frequent occurrence? Does it occur in one type of case more often than in another? If such invasion does occur, what are the most probable foci of origin and what part do they play in the death of the patient? Do bacteria migrate from foci or from the gastrointestinal or respiratory tracts after death and if so how soon? There are as many variations to the question of dissemination of bacteria after death as to the ones regarding terminal or agonal invasion of bacteria.

Because of the foregoing difficulties the few workers in the field who have reported the results of their efforts have reached various conclusions. Some support the reliability of the results, others believe that invasion of the blood and organs either before or after death renders the results valueless.

Many of the earlier students of this subject considered that postmortem cultures were of little value. Wurtz and Herman, Hauser and Birch-Hirschfeld found *Bacillus coli communis* to be a common invader of the blood stream and internal organs after death. They concluded that dissemination of bacteria from certain organs, particularly from the gastrointestinal tract, takes place soon after death, and because of this the results obtained from postmortem bacteriology were unreliable. It is noteworthy that in none of the cases which they mentioned was necropsy performed less than ten hours after death.

Fredette, although he admits the possibility of invasion after death, believes that cultural results obtained a few hours after death are fairly reliable in demonstrating the presence of organisms at the time of death.

Canavan and Southard, Landstrum and Austerlitz, and Monod and Macaigne concluded that the heart's blood, liver and spleen were seldom the seat of terminal invasion by bacteria of the intestinal tract, and that the bacteria found were of intravital occurrence, at least during the few hours immediately after death.

Flexner has pointed out that secondary or terminal infections are relatively common, particularly in chronic diseases.

Löw, Canon, Achard and Phulpin, and Gwyn and Harris, all agree that postmortem cultures should be made as a routine.

Giordano and Barnes, in 1922, thoroughly reviewed the literature and reported their results from a careful bacteriologic study of 213 postmortem examinations. They concluded that the bacteriologic changes noted may strengthen, illuminate, or sharply modify the cause of death, as revealed by clinical diagnosis. They admit the possibility of terminal invasion. Its occurrence must not be dismissed lightly as it may be the most important contributory factor to the cause of death.

Our results were obtained by working on the problem consecutively and independently for two and a half years. Each worker used a similar technic. Cultures were taken when possible from the heart's blood, liver and spleen, and from other organs when indicated. Accumulations of fluid and purulent material were also cultured. Blood from the heart was obtained usually from the pulmonary artery by means of a sterile syringe and needle. The surface of the artery was always seared either by means of a heated spatula or a projected flame. The surface of the organs to be cultured was seared in the same way and

the material was obtained through this sterile field by means of a special glass pipette or a metal loop. Pus and other fluids were obtained by aspiration or swabs. The utmost care was observed to eliminate contamination. Rosenow's dextrose brain broth and the ordinary blood-agar plates were inoculated as a routine with the material obtained for culture. As occasion demanded, special mediums were inoculated. All mediums were inoculated within one hour after the material to be cultured had been obtained. For comparison and control, direct smears were made from inflammatory surfaces and from the contents of abscesses. These smears were then stained and examined for organisms.

The value of our work was enhanced by the fact that the bodies had not been subjected to refrigeration and that the cultures were obtained within one to twelve hours after death, most of them within three hours. Table I shows that the ratio of positive to negative cultures more or less corresponds throughout. The three positive cultures of the third worker and the one positive culture of the first worker taken twelve hours after death were obtained from cases in which there was extensive infection prior to death. One case was that of septicemia, and from the extent of the infective lesions in the other cases terminal septicemia might have been present. We concluded, therefore, that at least twelve hours may elapse between death and postmortem examination without affecting the reliability of bacteriologic studies. This agrees with the view of Fredette who believes that cultures obtained a few hours after death are fairly reliable in demonstrating organisms present at the time of death. Giordano and Barnes also concluded that invasion of the blood stream rarely occurred and that sustained progressive increase in positive results at successive hours after death did not occur. In their series they covered a period of twenty-three hours after death.

TABLE I

POSITIVE AND NEGATIVE CULTURES OF HEART'S BLOOD; TIME OBTAINED AFTER DEATH

HOURS	FIRST WORKER		SECOND WORKER		THIRD WORKER		FOURTH WORKER	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
1	22	41	11	18	13	45		11
2	27	52	17	27	8	40	8	19
3	6	20	12	13	9	22	1	9
4	2	13	5	4		5	2	4
5		3	3	4	6	4		2
6		3	1			8	1	1
7	2	2	2	1	2	2		
8	1	2			2	10		
9	1			1	2	2		
10	1	1	2	2		1		
11		1				1		
12	1				3			
Total	63	138	53	70	45	140	12	46

On the other hand, Birch-Hirschfeld cultured the internal organs and heart's blood of twenty cadavers ten hours after death and found *Bacillus coli* in all of them. Wurtz and Herman also found the bacillus in the liver, spleen and kidneys of cadavers cultured twenty-four to thirty-six hours after death.

The total number of blood cultures made at postmortem and the total number which were positive and the percentage of positives are shown in

Table II. These data, when compared with those of other workers are low except those of the second worker; in this group there was a much higher percentage of cases in which there were definite foci.

The percentage of positive blood cultures obtained after death by other investigators is as follows: Fredette 35, Simmonds 48, Gradwohl 44, Canavan and Southard 44, Richey and Goehring 29, and Giordano and Barnes 38.8. Strauch, in 2000 cultures found 1001 cultures sterile. The cultures were obtained from the heart's blood at necropsy on an average of ten to fifteen hours after death. A marked variation may also be noted in the percentage of positive cultures obtained in our series. It can be easily understood that the type of cases dealt with by each worker must have a great deal of influence on the results obtained. For instance, a preponderance of cases in which there are infectious foci will raise the percentage of positive blood cultures. In only one case in our combined series did we find a positive blood culture without definite foci.

We considered that foci of infection were present in all cases in which the lesion was demonstrated pathologically, such as cases of peritonitis, abscess, meningitis, pneumonia with abscess formations, and ulcerative colitis. By comparing the percentage of cases with definite foci with the percentage of positive blood culture (Table II) a uniformity of results may be seen except in

TABLE II

CASES WITH POSITIVE BLOOD CULTURES AND DEFINITE FOCI

	BLOOD CULTURES			DEFINITE FOCI OF INFECTION		DEFINITE FOCI OF INFECTION WITH POSITIVE BLOOD CULTURES	
	NUMBER	POSITIVE	PER CENT POSITIVE	CASES	PER CENT	CASES	PER CENT
First worker	201	63	31.3	92	45.8	62	67.4
Second worker	123	53	43.7	90	73.2	53	58.9
Third worker	185	45	24.3	88	47.6	45	51.1
Fourth worker	58	12	20.7	31	53.4	12	38.8

the case of the first worker and the fourth worker. The discrepancy in the former group can be explained by the fact that, although the percentage of cases with definite foci was small in comparison with the others, yet the percentage of positive blood cultures was high, thus raising the total percentage of positive blood cultures. In the latter group the percentage of cases with definite foci was high with a low percentage of positive blood cultures, thus decreasing the total percentage of positive blood cultures. A study of the necropsy records showed the variation of percentage of positive blood cultures in cases with definite foci to be dependent on the severity and extent of the lesion present.

A list of simultaneous cultures of heart's blood, spleen and liver and of positive cultures was made in an attempt to determine whether cultures from any one organ indicated better than those of another the presence of bacteriemia. As this list only included cases in which all three organs had been cultured simultaneously, we were able to study the behavior of each organ to the bacteria present in the same case. Our data seemed to show that the

blood was a better indicator of bacteriemia than either the spleen or the liver.

Because of the generous blood supply of both liver and spleen it might be expected that if a positive culture was obtained from the blood itself, the organs also should contain similar bacteria. We found, however, that this did not occur (Table III). Whether this is due to inhibitory or bactericidal influences of these organs, we are unable to say. Cultures from the liver were more often positive than those from the spleen. This may mean that the fluids of the spleen are more bactericidal than those of the liver, or that the liver is more liable to involvement through its portal circulation in cases of peritonitis or gastrointestinal lesions.

TABLE III

A COMPARISON OF CULTURES OF HEART'S BLOOD, SPLEEN AND LIVER MADE SIMULTANEOUSLY

	CULTURES OF HEART'S BLOOD, SPLEEN AND LIVER	HEART'S BLOOD, SPLEEN AND LIVER POSITIVE	HEART'S BLOOD ALONE POSITIVE	SPLEEN ALONE POSITIVE	LIVER ALONE POSITIVE
First worker	155	31	6	4	2
Second worker	83	11	16	1	6
Third worker	10				2
Fourth worker	41	3	2		

Our results are in direct opposition to those of Giordano and Barnes who in comparing cultures of the blood and spleen made simultaneously concluded that the spleen serves as well, if not better, than the heart's blood for determining terminal bacteriemia.

A careful survey was made of all cases which were considered after postmortem examination to be of nonbacterial origin, such as brain tumors, diseases of the thyroid gland, cardiovascular diseases, and blood dyscrasias. The cases of this type studied by each worker were 109, 33, 97, and 27 respectively. In only one of these was a positive culture obtained from the heart's blood, liver or spleen. The time that the cultures were made varied between one and twelve hours after death. Postmortem invasion had not occurred. In the one case in which positive blood culture was obtained the diagnosis before death and at necropsy was acute lymphatic leucemia. At necropsy hemolytic streptococcus was found in the heart's blood, liver and spleen, but infectious foci were not demonstrated. Necropsy was performed ten hours after death. The patient, for four months before death, had been subject to colds and his recent illness was of two weeks' duration in which time he ran a temperature as high as 104°. Septicemia was not suspected and blood cultures were not obtained before death. It cannot be proved that septicemia was present but considering the course of the terminal illness and the bacteriologic changes noted at postmortem examination, this might have been the case.

SUMMARY AND CONCLUSIONS

1. This paper is based on a study of 567 postmortem examinations. The bacteriologic work was carried out by four persons working independently but consecutively.

2. The cultures were taken from unrefrigerated bodies one to twelve hours after death.

3. It was found that the lapse of twelve hours after death did not affect the results.

4. The percentage of positive postmortem blood cultures obtained by each worker was: 31, 43.7, 24, and 20.7 respectively.

5. In all but one case in which cultures of blood were positive definite foci of infection were demonstrable.

6. It was found that the number and type of cases in which infectious foci were definitely present was in marked relationship to the percentage of positive blood cultures.

7. By comparison of simultaneous cultures from the heart's blood, liver and spleen it was concluded that the blood is a better indicator of terminal bacteriemia than either the liver or spleen.

8. In all but one case of nonbacterial diseases, the cultures were entirely negative.

9. From the uniformity of results, it is believed that postmortem bacteriology is reliable and, if carried out carefully and as a routine, often leads to the solution of the exact cause of death.

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THE BIOLOGIC CHEMISTRY OF SKIN DISEASES AND SYPHILIS*

By C. N. Myers, Ph.D., Brooklyn, N. Y.

DURING the early ages the association of chemistry and medicine was illustrated by the efforts of the alchemist in his endeavor to prepare the elixir of life. On more stable foundation in the early part of the sixteenth century appeared that eccentric genius, Paracelsus, who looked upon medicine from the point of view of chemistry. Later there is found van Helmont, the originator of chemical physiology. Many other chemist physicians occupied chairs in the universities and contributed in a large measure to medicine and chemistry. The pioneer and epoch results came in 1828 when Woehler and later Liebig removed the barrier which was believed to separate organic and inorganic compounds, the chemistry of living things from the inert. Here the beginning of the study of life and its processes became related to the chemistry of so-called inert matter. For instance, Sir William Prout discovered the presence of hydrochloric acid in gastric contents, thus placing a vital relationship with the chemistry, physiology, and pathology of digestion. The work of Pasteur is well-known to all of us. The association of gout and uric acid likewise was also one of the early achievements. Thus it is seen that the chemical examination of the blood of living beings afforded methods of diagnosis which in turn has developed research on new substances and the presentation of methods of greater accuracy. In relation to the report to be presented in this paper, the investigations of Frederick William Pairy who devoted much of his time to the study of diabetes never saw the fulfillment of his vision of his research, the discovery of insulin. He however, so carefully described cyclic albuminuria that a rich heritage for later investigators was placed at their disposal. In this case again it appeared that too much time was being spent on an incurable malady, yet time, energy and vision conquered the apparent insurmountable obstacle and the time was not wasted. Thus in a wider sense and in a wider field the study of living cells and processes has given us a host of great biochemists, such as Pasteur, Wurtz, Hoppe-Seiler, Abderhalden, Schmidt, Emil-Fischer, all of the nineteenth century. In this connection the pioneer work of Ehrlich should not be overlooked; for in his endeavors he founded a broader science, chemotherapy, which correlated the best that is in chemistry, biology, physiology, pharmacology, and medicine. The names of living biochemists who have surpassed the work of the forefathers are a legion, and through their efforts centers of biochemical research have been established and the progress of medicine has been enhanced by their efforts.

The newer biochemistry does not restrict itself to chemical constitution but includes the physicochemical aspects of the living process and through these interpretations based upon exact laws, valuable aid in the diagnosis, prognosis, and treatment of disease is attained. It is however equally important

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that these newer aspects should not so engross the attention of the investigators that the older problems are neglected or overlooked. Examinations of greater and greater accuracy have been demanded and it is pleasing to say that the application of an exact science has furnished the necessary measures.

Sometimes the spark of life burns brightly, at others it glows dimly and yet through these diversities chemical methods are able to throw light on our bodies which act as simple furnaces. If one gland or another is in a stage of hypo- or hyperfunction, if some special enzyme or hormone fails in its activities, these derangements manifest themselves in the form of disease. Thus an insight must be obtained by chemical means which aids the physician in diagnosing and treating this symptom. Thus glycosuria, albuminuria, uremia, icteric index, basal metabolism, cholesteremia, uric acid, acid-base equilibrium, all offer much useful information as to functional activities of certain organs. Through physiology and pharmacology the relation of vagotonic and sympathetic overbalance add to our knowledge of permeability, and electronic exchanges in the body. Likewise, the protective mechanism of one individual as compared with another is closely if not entirely related to the chemical differences invested in these living operatives. Furthermore I for one believe that certain maladies malignant in nature can be traced to chemical origin. The researches of biochemistry can without question throw light on morbid processes and with this light producing therapeutic products through whose administration relief from suffering, may be obtained.

No better illustration of this idea can be given than in the use of salvarsan in the treatment of syphilis, the application of gold in certain diseases of the skin, insulin in the treatment of diabetes, mercurials in the reduction of edema, novocain in surgical anesthesia. These are but a few of the coordinative studies of the nature of disease. Thus it is necessary for the biochemist to familiarize himself with disease, its symptoms and lesions, as well as for the physician to become acquainted with the basic principles of physics and chemistry as exact sciences. For example, metallic substances may form coordination compounds with trivalent arsenic and antimony compounds of the arsenobenzol type by virtue of their residual affinities. The therapeutic effect is enhanced through the presence of these nonionic colloidal metals. This is illustrated by silver-salvarsan in which the silver oxide is protected from aggregation by the emulsoid action of the parent arsenical. Here physical and colloidal chemistry finds a common meeting point with the production of a more highly active therapeutic substance.

Our rapid progress in the more exact method of measuring functional changes in the living organisms has been brought about by the honest endeavor of groups of individuals exchanging ideas and then applying them to diseases in question. Their efforts along this line have found application in the study of skin diseases or those which may produce cutaneous manifestations. Looking at it broadly disease is only a manifestation in which the forces of the invader have succeeded in overcoming the powers of resistance of the invaded and as a result, our studies have endeavored to measure the destructive manifestations visibly and obscurely left in the field after the attack.

In order to develop a therapeutic measure it is primarily necessary to know the abnormalities produced by the disease in question. In the second

place it is then necessary to devise compounds and their derivatives which will produce changes similar in nature to those which are missing. These abnormalities may manifest themselves by the absence of certain definite substances or by an excess of these same entities, or still by functional changes which in some instances are more difficult to observe.

In our investigations of skin diseases the application of the well-known principles readily placed themselves at our disposal.

For instance, eczema, a very common disease easily recognized by all, showed very common physical manifestations, namely, an abnormal passage of fluid outward through the skin. This observation implied an abnormal regulatory phenomenon involving a semipermeable membrane. Regulation is the conservative method necessary for the free and independent life of the organism governed by necessary stimuli and reactions that result through this multiplicity of mechanisms which lead to phenomena designated as "life." In all physical and physiologic processes the problems of volume, time, temperature, and concentration enter into these exchanges of fluids through the cells and are governed by the complex principles of catabolism and anabolism associated with electronic exchanges. The cell, its wall, and its contents involve the more detailed study of dynamic equilibrium, not static equilibrium, dysfunction, or perversion of the normal regulatory processes leading to certain pathologic conditions of disease. Microscopic examination of cutaneous cells showed the presence of edema which is only a disturbance of the volume of the cell. This volume change of the cell could be interpreted only as a vital effort in the restoration of the electronic and osmotic balance.

With these ideas in mind our attention was directed to the more common skin diseases such as eczema, urticaria, psoriasis, acne, and the common dermatoses. In order to obtain the greatest number of values at any one interval it was necessary to fix the specimen so that it would not deteriorate and to obtain sufficient quantity so that a series of substances could be obtained simultaneously. It is our belief that for the first time our investigations have shown the importance of the chloride-sugar ratio. In carrying out these examinations the following substances are determined as an aid in diagnosis, sugar, chloride, urea, uric acid, cholesterol, and icterus index. This can be carried out in battery style and the report available within a few hours after the specimen has been obtained. No specimens are used unless immediately fixed inasmuch as investigation has shown that blood from the ice box is unsatisfactory for sugar and urea estimations. All the methods employed are checked by means of the addition of known amounts and recovery must be at least 95 per cent accurate. In other words, adequate control on the methods and the solutions employed is essential. The normal values established by us are in terms of whole blood: sugar 90 to 95, chlorides 445 to 455, urea nitrogen 14.5 to 15.5, uric acid 2.7 to 3.2, icterus index 4 to 6, cholesterol 150. During the past year about 40,000 determinations have been made and through these studies it has been shown that a single set of determinations is almost useless. For this reason a weekly examination, sometimes more frequently if necessary, is made on every patient under treatment.

The various skin diseases previously enumerated and syphilis which occasionally shows cutaneous manifestations are accompanied by marked disturbances

in the functional equilibrium of the organs of the body. For instance, the injection of arsenic preparations occasionally provokes marked disturbances of the involuntary nervous system. This disturbance can be measured by a study of the decrease of leucocytes in the peripheral blood and the accumulation in the splanchnic region. Furthermore psychic irritations (fear, joy, etc.), changes in temperature produce a change of the forms of activity of the skin, the skin becomes pale because of the involuntary contraction of the skin vessels. These changes cause the skin to become less permeable, water elimination through the skin is decreased as well as the electrical conductivity. This process is transient and reversible. When hypoglycemia exists, the skin vessels involuntarily become dilated, and the endothelial cells become highly permeable with an intensive perspiration.

With these physiologic principles in mind examination of individuals suffering from these diseases is carried out with the result that in eczema there are three well-defined groups of cases illustrated by the following classification:

a. Metallic group showing a low chloride and a high sugar. Our investigations on this hypochloremia and a hyperglycemia have been confirmed by many clinicians. For instance, arsenic intolerance, mercury intolerance readily produce this condition, and the estimation of the metal easily confirms this finding. Values for sodium chloride in these conditions usually lie between 300 and 400 mg. per 100 c.c. of blood and the accompanying hyperglycemia is 125 to 200 depending upon the severity of the condition. The values for urea, and uric acid are seldom disturbed in the group.

b. Hyperglycemia group closely associated with diabetes without the presence of sugar in the urine.

c. Protein group with high uric acid, moderately elevated or depressed urea with an increased icterus index.

These groups have been arrived at through the examination of more than 2500 patients and approximately 100,000 examinations. In psoriasis which has been under treatment, the biologic values for the most part fall in group A. Acne, furunculosis, and infections usually show a hyperglycemia and an increase in the uric acid values. Psoriasis is also accompanied by a hypercholesteremia. Details showing the classification of the patients by age, sex, color, etc., will be published in the clinical discussion of these values.

In syphilis, the patients showing an intolerance have a hypochloremia, a hyperglycemia, a decreased urea, and a high icterus index. The values are a good indicator of alarming disturbances which may follow, and therefore treatment should not be continued. These findings emphasize the use of drugs that are highly active in small doses rather than depending on low toxicity and large doses. Low toxicity and low therapeutic value go hand in hand. Therefore it is necessary under these conditions to increase the dose to obtain clinical results which lead to late by-effects. Furthermore, it shows that all of these conditions are closely associated with changes in the vagotonic and sympathetic balance which bring about alterations in the metabolism which can be measured by the substances mentioned above.

In conclusion it should be stated that chemistry and medicine are closely associated and by means of chemistry biologic changes may be accurately measured, thus affording valuable data in the diagnosis and prognosis of

pathologic conditions. Biochemical methods also afford an accurate means of following the progress of treatment as well as furnishing precautionary measures before instituting treatment either in skin diseases or syphilis.

For example, experiments have demonstrated that the permeability of the vascular endothelium of the splanchnic region always shows clearly a reaction opposite to that of the peripheral organs and tissues of which the skin is only a part. The examination of the blood by means of biochemical methods is closely associated with clinical aspects. Pruritus is invariably associated with hyperglycemia, edema with disturbances of the salt balance, recurring carcinoma with hyperglycemia and overweight, nephritis and hyperchloremia, eczema with hyperglycemia, hypochloremia, and hyperuricemia, syphilitic disturbances with sugar, chloride, icterus index, and urea disturbances.

By means of systematic accurate routine examination of the blood of patients many preventable clinical symptoms may be avoided. There must be a determination to obtain the specimen absolutely fresh, so that it is as near as possible to the clinical condition, otherwise the results are useless. It is believed that dermatitis, dermatitis exfoliativa, and jaundice can always be detected long before their manifestation in syphilitic patients, and thus avoidable. Metallic intolerance in association with metallic therapy can likewise be detected by a hyperglycemia, and a hypochloremia, in other words that is a numerical decrease in the sugar chloride balance (1 to 5 normal). As a result of many thousand determinations a complete blood examination is essential as a routine procedure in dermatologic clinics as well as general clinical practice.

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EFFECT OF COFFEE AND TEA ON GASTRIC SECRETION*

By W. Horsley Gantt, M.D., U. S. S. Russia

THE almost universal use of coffee and tea as beverages among civilized people is an important reason why we should thoroughly understand their action. In many European countries indeed they furnish the main water intake, especially in Russia where the drinking of quantities of weak tea is a habit prevalent among all classes.

The purpose of our experiments was to find out the chemical action on the secretion of the stomach of coffee and tea when taken with food.

As is well known, the secretion of the stomach to any substance which is eaten consists of two components: the "psychical" or nervous secretion, and the chemical secretion. This was shown first in 1852 by Bidder and Schmidt and later by the sham feeding experiments of Pavlov.[1] As the nervous secretion, being a conditioned reflex, varies with each individual according to whether he likes or dislikes tea or coffee and with many other factors, we desired to eliminate this so as to get the pure chemical or unconditioned effect.

*From the Physiological Laboratory of Prof. Pavlov at the Institute of Experimental Medicine, Leningrad.

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TABLE I

COMPARISON OF TEA AND WATER ON GASTRIC SECRETION

	150 GM. DISTILLED WATER PLUS 100 GM. BREAD INTRODUCED INTO GASTRIC FISTULA				150 GM. TEA PLUS 100 GM. BREAD INTRODUCED INTO GASTRIC FISTULA				
Order of experiment	1st	4th	7th	Average	2d	3d	5th	6th	Average
Latent period	15 min.	11	—	13′	—	16′	10′	15′	13:49 min.
First hour	3.0 c.c.	3.0 c.c.	2.4 c.c.	2.8 c.c.	2.4 c.c.	2.4 c.c.	2.6 c.c.	3.5 c.c.	2.8 c.c.
Second hour	1.7	2.1	0.9	1.57	—	1.6	1.0	2.0	1.53
Third hour	1.4	2.1	—	1.75	—	0.9	1.9	2.0	1.6
Fourth hour	0.7	1.6	—	1.15	—	0.8	1.6	1.5	1.3
Total				7.9 c.c.					7.3 c.c.

TABLE II

COMPARISON OF COFFEE AND WATER ON GASTRIC SECRETION

	150 GM. DISTILLED WATER PLUS 100 GM. BREAD INTRODUCED INTO GASTRIC FISTULA						150 GM. COFFEE PLUS 100 GM. BREAD INTRODUCED INTO GASTRIC FISTULA					
Order experiment	1st	2d	5th	7th	9th	Average	3d	4th	6th	8th	10th	Average
Latent period	24	24	32	30	21	26 min.	15	29	29	25	22	24 min.
First hour	1.1 c.c.	0.8 c.c.	1.0 c.c.	2.0 c.c.	1.6 c.c.	1.3 c.c.	2.0 c.c.	1.2 c.c.	1.6 c.c.	1.6 c.c.	1.7 c.c.	1.6 c.c.
Second hour	0.6	0.4	0.6	0.8	0.5	0.58	0.9	0.8	0.7	0.6	0.6	0.7
Third hour	0.5	—	—	0.9	—	0.7	1.6	1.5	—	1.2	1.0	1.07

We first tried to introduce the mixture of bread and tea into the stomach through a stomach tube. Here we met with several difficulties: the food passed slowly along the tube and often clogged; it was difficult to prevent the animal from seeing the food and tasting it when the tube tip was pulled out over the tongue; the introduction of the tube evoked the passive-defensive reaction in the dog, which might have a disturbing influence on the gastric secretion.

Our dog had already a stomach pouch, according to Pavlov's method, so that the secretion could be collected and measured accurately. In order to overcome the above-mentioned difficulties, we made a gastric fistula in the large stomach, and began our experiments about three weeks after operation when the secretion had returned to normal.

Our dog was about three years old, and had been used in the laboratory for more than a year previously, having shown a constant and regular gastric secretion.

The experiments were done as follows: no food was given the morning of the experiment, the last feeding being on the previous evening at 6 P.M. The stomach was first washed clean with ordinary water at room temperature; if there was any secretion we waited until it disappeared or until the contents of the stomach became alkaline. The dog was put on its back, and the bread and tea mixture was introduced through the gastric fistula. Every precaution was taken to keep the animal from seeing and smelling the food in order to prevent any psychical secretion.

The dog was returned to the stand, the latent period noted by testing with litmus the reaction in the stomach pouch, and the gastric juice therefrom collected and measured every fifteen minutes.

In order to eliminate any daily variation in the secretion, control experiments were done on the alternate days, using a mixture of bread and a volume of water equal to that of the tea. Both the tea and coffee were made very strong by boiling for five minutes 3 to 5 gm. of tea or 5 gm. ground coffee with 150 c.c. of water. Three experiments were done in a week on alternate days.

The results of our experiments are shown in Tables I and II. (These tables are not to be compared with each other, as the experiments with coffee and tea were done at an interval of several months.)

DISCUSSION

In the human, Miller, Bergheim, Rehfuss and Hawk,[2] compared the action of 1,000 c.c. of coffee or tea with water. This amount of coffee and tea plus the test meal of rice, they found, produced nervous symptoms such as palpitation, flushing, shivering, etc., and delayed the secretion, but finally the same acidity was reached as from the test meal alone (cited by Babkin[3]).

Kestner and Warburg[4] found in a dog with a duodenal fistula that coffee produces a slight gastric flow and tea very little.

Kestner and Knipping[5] state that the action is due only to those substances entering into solution, i.e., 25 to 30 per cent of the weight of roasted coffee. In 25 gm. of a watery extract made from 100 gm. of ground coffee there are,

besides other things, 1.7 gm. of nitrogenous compounds, about 1.5 gm. of which is caffeine; also 5.2 gm. of oil and 4.1 gm. of mineral substances.

Thirty to 40 per cent of the weight of tea enters into solution, and 2 per cent of the resulting solution is caffeine. Thus in a cup of coffee made from 7.5 gm. of coffee there is 0.1 gm. caffeine, but in a cup of tea prepared from 2 gm. of tea leaves, only 0.02 gm. caffeine. These authors remark that nothing of nutritive value is present, neither albumin nor vitamines, and that their effect comes from the action of the caffeine on the nervous system and on the gastric secretion. Bickel and Eweyk,[6] however, state that the influence on the stomach secretion is not from the caffeine but from the products arising during the process of roasting.

The use of coffee and tea as palatable beverages taken with food should not be overlooked. In this capacity they may aid digestion in two ways: First, by the stimulation of the "psychical" or nervous juice, and second, by adding to the water intake. That the nervous secretion may be considerable has been well established by the sham feeding experiments carried out in the laboratory of Prof. Pavlov.[7] The gastric juice obtained by five minutes of fictitious feeding in a dog with an esophageal fistula so that none of the food enters the stomach, but is only swallowed and dropped out from the fistula) may last for three hours and almost equal that produced by bringing the food directly into the stomach through a fistula, i.e., the chemical secretion.

As regards the water intake on the flow of gastric juice, Prof. Pavlov says: "If tea and coffee through their water content help to supply a water deficiency in the body, they may become extremely important factors in digestion. In my experiments with sham feeding it was shown that if the animal was deprived of fluid the gastric secretion gradually sunk, and after several days became zero. As soon as water was given, the secretion began. If the water intake is too low all of the secretions will be greatly decreased." (Personal communication for this paper.) Of course, from this point of view, coffee and tea have no specific value, and are of use only in so far as they insure a sufficient amount of water for digestion, and they could be substituted by any other aqueous beverage provided it were taken in the same quantity.

SUMMARY

The chemical action of a mixture of tea and bread on the stomach secretion was found to be practically the same as a mixture of bread with an equal amount of water. A mixture of coffee and bread produced a slightly greater amount of gastric juice during the first two hours. The latent period (the beginning of the secretion) was not affected in either case. The increase in juice was very little, 0.3 c.c. for the first hour in the stomach pouch, or about 3.0 c.c. for the whole stomach. The nervous element was eliminated by introducing the mixture through a gastric fistula, and the collection of the juice was from a miniature Pavlov's stomach.

We may conclude that the effect of even very strong coffee and tea on the stomach secretion depends almost entirely upon the individual, i.e., upon the nervous secretion, and upon the water content, there being little or no chemical influence due to the tea and coffee per se.

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CALCIUM DEPOSITION IN TISSUES OF DOGS AND MICE BY THE AID OF PARATHORMONE*

By Aaron Learner, M.S., Chicago, Ill.

I. INTRODUCTION

THE development of a potent extract of the parathyroid glands ended a long search for a hormonal substance of this gland. This substance has given rise to many studies on calcium metabolism, the parathyroid glands, and to therapeutic studies.

It has been forty-eight years since Sandstrom (in 1880) first discovered the parathyroids, but for many years their great significance lay in the rôle they played in preventing tetany by their presence, in whole or in part, in the body. The work with these glands was related to describing the picture of parathyroid tetany. In later years, however, chemical studies of the glands and of the blood serum were undertaken, which led to the accumulation of the data now known and accepted more or less. At various times the explanation of the function of the parathyroids was attributed to a detoxicating action against guanidine and methyl guanidine, and then again by others to an influence in the regulation of the calcium metabolism, and by others still to a regulation of the acid base equilibrium with disturbance thereof in parathyroidectomy causing the symptoms of tetany.

While there is no fully adequate explanation of the modus operandi of the parathyroid glands, the influence of these glands on the concentration of calcium in the blood is given full recognition. Successful preparation of the parathyroid glands has been developed by Hanson, Beermann, Collip, and others. Injection of these preparations results in the development of a hypercalcemia with a train of symptoms, namely, anorexia, emesis, increasing somnolence, and phenomena of circulatory weakness. This has been described by many workers, but especially by Collip, whose preparation and standardization of the product known as parathormone is probably the most significant work of any in increasing our knowledge of these glands.

The most striking results of parathormone injection have been obtained on dogs. Subcutaneous or intramuscular injections, depending on the dose, may raise the blood serum calcium well over 100 per cent. Positive, though not so

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striking results have been obtained in calves, cats, and rabbits. These latter, though showing a hypercalcemia, are relatively resistant, for they do not develop the serious symptomatology attendant on the hypercalcemia in the dog.

Several questions are raised by these observations. What is the cause of the hypercalcemia, and what are the possible consequences of the increased calcium content? There is as yet no adequate answer to these questions.

Greenwald and Gross have hypothesized the existence of a calcium dissolving substance, which is either parathormone itself or a substance developed as a result of its action. This substance is necessary to keep in solution the excess of $Ca_3 (PO4)_2$ which Holt, LaMer, and Chown have shown to be present in the blood.

Stewart and Percival proceeded to answer the questions raised by the calcium raising power of parathormone, in a series of interesting experiments. They showed that parathormone raised the calcium in the blood serum even when the factor of any influence on absorption or excretion was eliminated by evisceration. They also reached the conclusion that the liver, spleen, pancreas, pituitary, thyroid, and central nervous system have no special function in connection with the effect of the parathyroids on the blood serum calcium content. They came to a conclusion which is in agreement with that by Greenwald, namely, that the calcium drawn into the blood comes from the soft tissues, or from the bone, or both.

There is that other question to be answered, as to the consequences of the hypercalcemia. We have mentioned the clinical picture which develops. Are there any pathologic consequences?

M. B. Schmidt in his discussion of pathologic calcifications divides the same into two main groups:

1. Calcifications occurring where there have been local tissue disturbances without any change in the calcium metabolism, and
2. Calcification occurring in healthy tissues as a result of changes in the calcium metabolism.

Important factors are:

a. Oversaturation of the blood and tissue fluids with calcium.
b. Inability on the part of the excretory organs to excrete calcium satisfactorily.
c. Changes in the solubility relations in the blood for calcium.

In the different studies on the chemistry of the process of calcification various conditions were considered as important. These were, (1) the formation of calcium soaps, (2) the combination in some way of calcium with phosphoric acid, (3) the physical adsorption of calcium salts, and (4) calcification as a simple precipitation. The last two gain most support now.

"Hofmeister advances the hypothesis (Wells' *Chemical Pathology*) that when the cartilage or other matrix becomes saturated with calcium salts, any decrease in CO_2 content of the solution will lead to a precipitation of calcium salts, thus restoring to the cartilage its power of absorbing more calcium salts whenever the fluid comes to it with a higher degree of saturation with calcium salts and CO_2. In support of Hofmeister's idea is the fact that a similar fluctuating effect, produced by alternately varying the reactions by feeding acids and bases, was found by Rabl to lead to metastatic calcification."

Rabl fed, alternating every two days, to a group of mice, first a diet yielding an acid ash, then a diet yielding an alkaline ash. At the same time he increased the calcium content of the food. His view was that the blood overloads itself with calcium when there is a reaction of acidity, then there is a precipitating out of the calcium with a developing alkalescence. Dreyfuss subsequently repeated the experiment of Rabl with some slight modifications, in that he had three additional groups, namely, acid ash diet alone, and alkaline ash alone, these having increased calcium, and a group maintained on the usual diets. Metastatic calcifications were found in all the groups, though to a lesser degree in the latter groups. Even in these, however, some calcification was found in the kidney and lung (in the lung the calcium being mainly limited to the bronchial cartilages). Dreyfuss urges caution in the interpretation of the results, and the transference of inferences to other animal types, and he states that calcifications in mice are not unusual findings under any circumstances.

Rabl's work lays stress on and supports the fact of the importance of alternating reaction. The question arises as to the possibility of calcifications in a healthy organism through simple overloading with calcium. Katase was able by simple injection (subcutaneous, intravenous, and intraperitoneal), without any previous tissue injury to produce calcifications in almost all tissues and organs. He used guinea pigs and rabbits.

Katase noted in addition to the calcification of the elastic membranes of the alveolar septae of the lungs some interesting findings in the bronchi. He found the bronchial mucosa more or less desquamated. The epithelial cells showed in their cytoplasm numerous inclusions of calcium granules, presenting a picture quite similar to that presented by mucous production. This picture he found to be identical with that of the colon mucosa. He found calcium granules in the lumen. He concludes, therefore, that the bronchial mucosa can serve a calcium excreting rôle, and continues to say that if the calcium granules came from calcified lung masses, there should appear in the sputum incrusted tissue elements, and the lungs should also show evidence of breaking down. The significance of bronchi as excretory organs for calcium was first established by these experiments.

In the intestine the calcification was in the connective tissue muscularis, muscularis mucosae, and in the membranae propriae of the glands.

In the liver the calcified liver cells were in groups of different sizes, and were found in almost any part of the acini in different places. There is as it were a network of calcified liver columns.

Of the tissue elements of the kidney, the epithelium of the convoluted tubules suffered most, and then the interstitial tissue as the membranae propriae. The explanation offered for these results is, that so long as the calcium is excreted in a normal fashion there is no calcification, but that when the excretion is interfered with, or the calcium content of the body fluids exceeds the excretory capacities of the kidney and intestines, then there results a precipitation of calcium in the different tissues and organs.

Thus we see that calcification can occur after injection of calcium salts. In parathormone we have an agent that can mobilize calcium. Hueper reported the findings of metastatic calcification in dogs by its use.

We have repeated the experiment, using 100 to 150 units parathormone, Collip, with results that are to some extent similar to Hueper's. We, however, have some very interesting additional findings to report. It was interesting to note the tendency of the calcium to be deposited at different sites of election in the different organs.

II. EFFECT OF PARATHORMONE ON DOGS

Since our chief aim was to study the effect of parathormone on mice, we used only two dogs as controls. The dogs developed a hypercalcemia of 16.77 and 19.57 mg. per 100 c.c. respectively. The latter dog showed very pronounced calcium deposition, while the dog with the smaller rise showed a lesser degree of calcium deposition.

Macroscopically, little was to be seen, except that the blood was clotted, and that the stomach showed evidence of a spastic state by the marked rugae

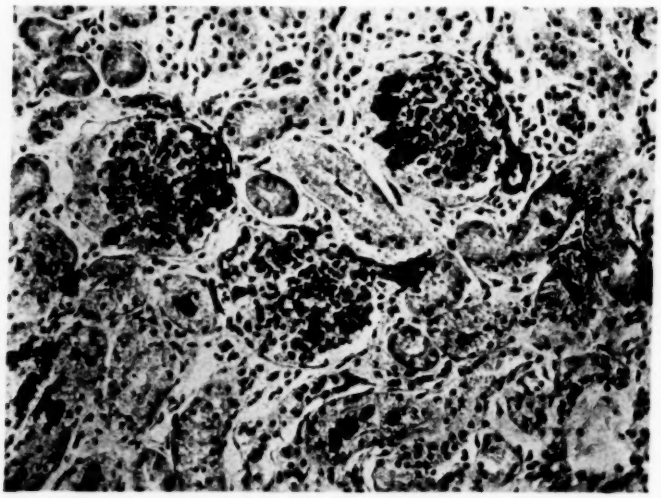

Fig. 1.—Kidney from a dog. (Kossa stain, counterstained with Hemalum Eosin.) Calcium shows up black, and is seen in Bowman's space, in the lumina of some of the tubules, and in some of the epithelial cells. A calcified artery is seen, and some calcium is present on the basement membrane of some of the tubules.

formation. The stomach and intestinal mucosae appeared hyperemic, but there was no evidence of extravasation of blood.

The Kidney (Fig. 1) showed on microscopic examination the following:

For the most part Bowman's capsule appeared as an unaltered, distinct single cell layered epithelium. The cells of the glomerules were of good staining quality. A few of Bowman's capsules showed calcium deposits. Much calcium, however, was found in Bowman's space, and between the loops of the glomerular tufts. There was a granular débris in some of the Bowman's spaces.

The tubular epithelium where there was no deposition of calcium showed no alteration from the normal staining qualities. Where, however, the tubular epithelium showed calcification, there it presented an appearance of necrosis, the change in the cells being directly proportional to the amount of calcium.

The calcium deposition in the epithelium proper varied from a few granules to a solid mass. Most of the epithelium, however, was unaltered. It was in the lumen of the tubuli where the calcium was most to be seen. The granular débris seen in some of the Bowman's spaces was present in some of the tubules. There was some calcium on the basement membranes of the tubules, and also in the walls of some of the arteries and capillaries.

The Liver (Fig. 2) Cells about the portal canals as a rule were unaltered; those centrally, however, showed a slight degree of atrophy, probably due to the passive congestion which was present. The calcium was deposited in the form of granules which were present in greater or lesser amount in the parenchyma cells and to a lesser extent in the Kupffer cells. The localization of the

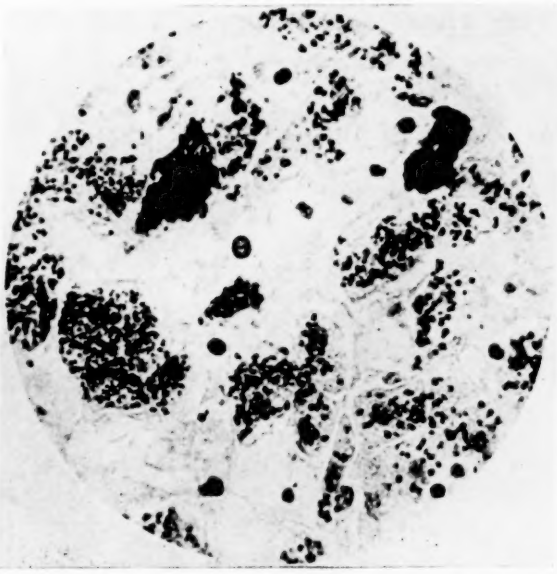

Fig. 2.—Liver from a dog. (Stained as Fig. 1.) Calcium granules in liver cells, and in a Kupffer cell.

calcium seemed to correspond to the areas where congestion was more marked, namely, centrally. In places the calcium was in the capillary walls adjacent to the liver cells.

The Lungs (Fig. 3) in addition to showing calcium deposits in the elastic fibers of the alveolar septa where it has already been described by others, showed a very marked deposition of calcium in the bronchial walls, on the elastic fibers and in the tunica propria of the bronchi. The bronchial epithelium showed no calcium in it, and it was desquamated. The bronchial cartilage was free from calcium. Small hemorrhages were noted.

The Heart showed considerable calcium deposition between the muscle fibers. The individual cardiac muscle fibers were seldom affected. The intima of the coronary vessels showed calcium deposition.

The Spleen was unaltered except for the deposition of calcium in the intima of the larger branches of the splenic artery immediately below the endothelial lining.

The Intestine including the stomach showed calcium in the basement membrane about the acini, in the vessels of the submucosa, and in and between the fibers of the muscularis. Calcium was present in many of the delomorphic cells of the stomach.

In the *pancreas* the calcium was in the interstitial tissue about the acini.

To summarize our findings in the dog, we noted calcium deposition in the following sites: In the kidney the calcium deposition was in the glomerular tufts, Bowman's space, the capsule, the lumina of the tubules, some of the tubular epithelium, the basement membranes of the tubules, and the arteries. In the liver the calcium was mostly in the liver cells in the centers of the acini, and to a lesser extent in the Kupffer cells. In the lungs the calcium was in the alveolar septa and below the bronchial epithelium, localized mainly in the elastic fibers. In the heart calcium was deposited mainly between the muscle

Fig. 3.—Lung from a dog. (Stained as Fig. 1.) Calcium deposition in alveolar septae, and beneath bronchial epithelium. No calcium in the cartilage.

fibers. In the spleen only the intima of the artery was affected. In the pancreas the interstitial tissue; in the intestine the connective tissue about the glands; and in the stomach the delomorphic cells showed calcium deposition.

COMMENT

Thus in the hypercalcemia produced by parathormone we get a series of pictures much the same as that of Katase's produced by injection of calcium. Especially interesting and differing from the others, however, is the picture of the kidney, wherein the arrangement of the calcium in the glomerular tufts, Bowman's spaces, and the lumina of the tubules gives morphologic proof of the manner of elimination of calcium from the kidney, namely, through the glomeruli. The findings of Greenwald and Gross that in dogs injected with parathormone there is an increase in the calcium and phosphorus in the urine,

emphasize the rôle the kidneys play in the elimination of calcium and phosphorus.

The explanation for the calcium deposition in the heart, lungs, and stomach is attributed to the fact of the increased tissue alkalinity consequent on the acids produced or eliminated in those sites.

The calcium deposition in the liver is of interest, for the liver, and especially the Kupffer cells are not common sites of calcification. The following may in part account for the calcification:

"Venous blood (Wells' *Chemical Pathology*) contains more diffusible alkali because the CO_2 combines with and tears away part of the bases that are held by the proteins, especially in the corpuscles, and so alkaline carbonates are formed and enter in the plasma."

Two important factors are thus seen to play parts in the calcifications noted. One is that of the altered solubility relations associated with an increase in the calcium, and the other is that which was expressed by Katase, that where the calcium content of the body fluids exceeds the excretory capacities of the kidney and intestine, then there results a precipitation of the calcium in the different tissues and organs. It is also possible that the underlying condition may be vicarious excretion, just as it is the case in uremia with the nonprotein nitrogen products.

III. EXPERIMENTS WITH PARATHORMONE ON MICE

The studies on the dogs with parathormone interested us in its effects on mice, especially as they are so prone to show spontaneous calcifications. We used 44 mice, which were subdivided into groups after the fashion indicated by the accompanying table:

		TOTAL NUMBER OF MICE			
GROUPS	DIET	AT START	SURVIVING	INJECTED	NOT INJECTED
I	Ca −	12	(8)*	8 (5)	4 (3)
II	Ca +	12	(12)	8 (8)	4 (4)
III	Ca + +	20	(20)	10 (10)	10 (10)
		44	(40)	26 (23)	18 (17)

*Numbers in ( ) indicate mice that were available for section.

In the injected groups of mice the amount of parathormone used was 1 unit each for the first seven injections, and 2 units each for the last five injections. In all a total of twelve injections and seventeen units of parathormone were received by the majority of the mice over a period of five weeks.

The diet of the mice through the period of the experiment was a diet developed by Osborne and Mendel with food of the general composition of

Protein	18%
Lactose Salt Mixture	28 -29.5%
Brewers' Yeast Dried	1.5- 2 %
Starch	25.5-27.5%
Butter Fat	18%
Lard	7%

Distilled water was used. The calcium free diet (Ca-) contained 0.008 per cent of calcium. The control food (Ca+) contained 0.546 per cent of calcium, and the rich calcium food contained double the amount of calcium (Ca++) in the control food. The lactose salt mixture was based on the composition of the salts of milk, and the ash of the entire mixture varies only slightly from neutral reaction.

A few of the mice died before the end of five weeks, but the majority were killed at the end of the five weeks, and pieces of the following tissues were taken for section: heart, lung, liver, kidney, spleen, adrenal, stomach, brain, and femur. The pieces were fixed in alcohol and stained by Kossa's method. As counterstain, hemalum or hemalum-eosin were used.

Microscopic examination of the organs revealed negative results except in the bronchial cartilages (Fig. 4). These were calcified in the majority of

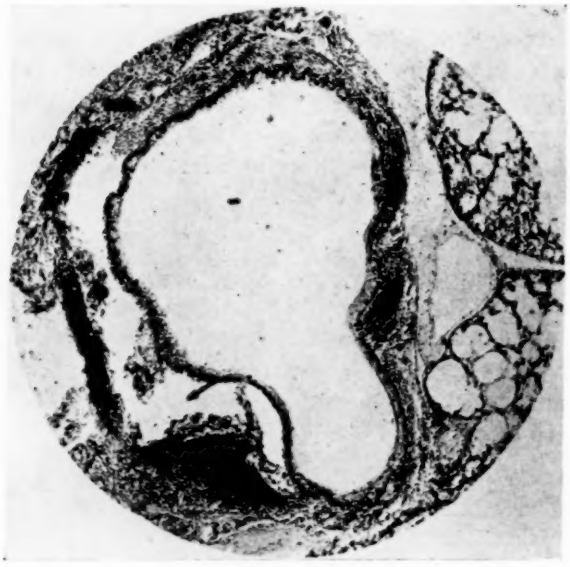

Fig. 4.—Lung from a mouse. (Stained as Fig. 1.) Calcium deposition only in bronchial cartilage. None elsewhere.

cases, varying in degree from slight to very much. It is to be noted that normally the bronchial cartilages of mice show a degree of calcification which though usually not very intensive, is nevertheless positive. In our material it seemed that the intensity of bronchial cartilage calcification was more marked in the injected than the uninjected mice. It seems thus that with parathormone and calcium, what occurs is an intensification of what is for mice an apparently physiologic process. These findings, however, cannot be stressed as significant from the standpoint of the calcium raising power of parathormone in mice, or as bearing any evidence for calcification in mice resulting from parathormone administration.

Several mice were also injected with 10 units parathormone. When one realizes that these mice averaged 20 gm. in weight, one readily recognizes what an incredibly large dose these mice received. Yet after forty-eight hours of

observation these mice manifested no signs whatsoever of any ill effects. Histologic examination supported the observations during life. No other calcifications than those of the bronchial cartilages were seen.

While we were making the histologic studies of the effect of parathormone on white mice, Süssmann reported some chemical studies on white mice with parathormone. He determined the normal blood serum calcium content of the white mouse to be about 18 to 20 mg. per cent. He then injected parathormone, Collip, in doses of 10 to 20 units, and was unable to notice any effects on them. He points out that a dose of 10 units for a mouse is the equivalent of a dose of 20,000 units for an adult of 64 kg., and he remarks that such dosage speaks for itself. Thus we see in Süssmann's studies chemical findings which support our histologic findings, for he was unable to detect any rise in the blood serum calcium content, or if there was a rise it was very insignificant.

Attention is called to the fact that the resistance of white mice to injections of such character is not new, for Voegtlin and Dyer have shown that white mice and also white rats are capable of enduring doses of epinephrin and pituitary extract 100 to 1000 times the usual dosage for other animals. To account for this peculiarity in the present state of our knowledge is an impossibility, and we must for the present pass over it vaguely by stating that the white mouse possesses an especial resistance to such hormonal substances.

Katase makes the observation that the intensity of calcification is inversely proportional to the normal calcium content. The calcium tolerance of each organ must vary. Quite possibly the organs with high calcium content already under physiologic conditions possess an especially high calcium metabolism and are therefore able to take care of a large influx of calcium without any resulting precipitations. The organs, on the other hand, with low calcium content may not be able to cope with a high calcium influx, therefore calcification occurs.

It may be that the findings of a high calcium content for the mouse speak for an active calcium metabolism, and an ability on the part of the mouse to handle a calcium increase without precipitation or evidence of rise in content. Further study is necessary.

SUMMARY

A histologic study of the experimental calcification in dogs produced by injections of parathormone gave the following results:

In the kidneys, the calcium is found in the Bowman's space of the glomeruli, in the lumina of the tubuli and, to a lesser degree, in the tubular epithelium. Some also is present in the wall of the smaller arteries. In the liver, calcium is deposited in the form of granules in the liver cells of the central portions of the acini and also in the Kupffer cells. In the lungs, calcium is observed in the wall of the bronchi below the epithelium and in the alveolar septa. In the heart, the calcification involves chiefly the interstitial tissue between the muscle fibers, while much less is found inside the muscle fibers. In the intestine, calcium can be detected in the connective tissue about the basal portions of the glands. Some of the delomorphic cells of the stomach are seen filled with calcium.

These microscopic findings are explained on the basis of either a fluctuation of the reaction with alteration in the solubility for calcium, or as the result of a simple overloading with calcium.

Mice injected with large amounts of parathormone show only an increase of the physiologic calcification of their bronchial cartilage. The otherwise negative histologic findings are in accord with the great resistance of these animals against parathyroid extracts.

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EPINEPHRIN ADDICTION IN BRONCHIAL ASTHMA*

By R. W. Lamson, Ph.D., M.D., Los Angeles, Calif.

IT IS a common custom to permit, or even urge, the chronic asthmatic to treat himself with epinephrin in order to relieve the severe paroxysms. If the patient is unable to give the injections to himself, some member of his household is usually trained to perform this service. It must be admitted that this procedure has spared the asthmatic much suffering, and not a little expense. Certain objections, however, are inherent in the method. These I shall attempt to point out with the aid of the following brief case histories.

Case 1.—Miss M., white female. Aged twenty-three years. Scotch ancestry.

Past History.—Patient dates the onset of asthmatic symptoms from a fall off a horse several years ago. This did not result in the fracture of any bones but she was confined to bed for a few months. It was said that she had injured her neck. More detailed questioning revealed that it was really two to four months after the injury that the first attack of dyspnea occurred. The onset did not appear to be associated with a respiratory infection or with any communicable disease. The subsequent attacks could not be correlated with season, climate, or other extrinsic factors.

Physical Examination.—The findings were typical of those in bronchial asthma. The patient was also poorly developed and nourished.

Sensitivity.—Allergic skin tests revealed a moderate degree of sensitization to significant pollens and to feathers.

Treatment.—She had been put on epinephrin therapy by her local physician, a year or more previously. The injections were given by her sister who was, therefore, obliged to be a constant companion of the patient. Specific pollen therapy was tried and at first benefit was obtained, but later it seemed to aggravate her condition.

Subsequent History.—Almost complete relief, for several weeks, was obtained when an aunt and uncle visited the patient. A few days before the departure of her relatives her asthmatic symptoms returned. One very striking incident, which occurred during this period, seems worth recording. The patient was acting as "sight-seeing" guide to her relatives on an automobile trip. The uncle was driving the car and the patient was busily occupied consulting maps, directing him which road to take, and pointing out and discussing points of interest. After a few hours of driving a site was selected for a picnic supper. Almost immediately after the car stopped the patient had a severe asthmatic attack. She had been entirely free during the ride and as this attack started before she got out of the car exertion did not seem to be the precipitating factor. Epinephrin was required to relieve her. No clue could be obtained to any environmental factor that might have caused the attack.

A day or two after her relatives had departed she was having nearly constant asthma. She had received no specific pollen therapy for several weeks. During the next two weeks I was called to her home several nights in succession, and always at about the same hour. Her sister had been giving her large amounts of epinephrin but the patient obtained little relief. A smaller dose out of my bottle of epinehrin would often relieve her, though occasionally it was necessary to resort to the use of opiates.

The amount of epinephrin had to be increased daily and its effectiveness seemed to decrease about as rapidly. Soon she was taking an ounce every three or four days in spite of which she would enjoy but short periods of freedom from symptoms. The large amount of this drug, though failing to relieve the dyspnea, seemed to bring about a lowered function of her several systems, especially the gastrointestinal tract.

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She was placed in a hospital and an attempt was made to reduce the total amount of epinephrin used by giving smaller doses of the drug, repeating more frequently if necessary. It was noted that the injections were repeated at nearly identical hours each day. This fact and the observation that at times smaller doses of the drug from my bottle would be effective, suggested the use of psychic therapy. This was started and hypodermics of sterile salt solution were substituted for the active drug.

The first night that epinephrin was withheld she was given a hypodermic of morphine sulphate 16 mg., and atropine sulphate 0.44 mg. These drugs had been tried before but had not effected a reduction in the amount of epinephrin. For the next few days it was necessary to repeat the hypodermics of salt solution at about the same intervals she had previously demanded the active drug. If the salt solution did not give satisfactory relief small doses of epinephrin were employed.

She was discharged within two weeks free from asthma, and she had used no epinephrin for several days previously and required but few hypodermics of salt solution during any twenty-four-hour period. The improvement in this case can definitely be dated from the time salt solution was substituted for epinephrin. Her sister was instructed how to continue the psychic therapy after the patient returned home. Her general condition, as well as her asthma, continued to improve and she was symptomless for several months without any form of therapy.

CASE 2.—S. G., white male, Irish ancestry. Patient gave his age to one physician as fifty-two to another fifty-four years. The actual age was sixty-two, and his general appearance was more consistent with the actual age.

Past History.—First attack of paroxysmal dyspnea occurred three years previously following a cold. No specific etiology was indicated by the history. The attacks seemed unrelated to season or climate, in fact they were nearly constant. He was forced to give up his business because of the severe asthma. Exertion often precipitated an attack, though he was willing to admit that he seldom had an attack when he was deeply interested in something, even though it did entail a certain amount of exertion.

Physical Examination.—The findings were characteristic of those in bronchial asthma, but emphysema was more marked than would be expected in one who had had asthma for so short a time. Cardiac findings at several examinations were not suggestive of any disturbance of the circulatory system. He was poorly developed and nourished.

Sensitivity.—Routine food, pollen and other allergens were tested by the scratch and intracutaneous methods and no sensitizations were demonstrated. He stated that at one time he had reacted to horse dandruff.

Treatment.—This patient had been using epinephrin for about two years and required frequent injections at night.

Subsequent History.—The patient's wife had been visiting in the East for several weeks. For about two weeks before the date she was expected to return the patient had been having very severe asthma. He had been in bed at home and required practically hourly does of epinephrin day and night. Often it was necessary to repeat a dose of 8 or 10 minims two or three times a few minutes apart. Two over-solicitous sisters were kept busy administering to this patient.

He was placed in a hospital in July, 1928. A short time after his admission he had a severe paroxysm. His condition was markedly aggravated because he did not receive the instantaneous service that he was accustomed to at home. This attack was controlled with difficulty. The next day he accused his physician and nurse of neglect and the hospital for having such poor service. For several days small doses of epinephrin were used and repeated in fifteen minutes if relief was not obtained. Frequently it was necessary to give a total of 23 to 25 minims in a period of thirty minutes before he would admit that he was relieved.

A study of his chart disclosed that certain hours each day appeared to be critical periods. These centered about the time his visitors left in the afternoon and also between 11:00 P.M. and 1:00 A.M. The reason for the latter period will be suggested later. For several days he averaged from 98 to 147 minims of epinephrin in twenty-four hours. Ten

minims was the maximum dose. Symptoms were getting definitely more severe, and he was advised that a change in the method of giving the drug might yield better results. He was informed that he would be given larger doses but at less frequent intervals.

At midnight that night he had a severe attack and was given 15 minims of sterile salt solution. Just before the injection he asked his nurse how much she was going to give him. He was told the amount but not the contents of the injection. He protested against such a large dose but the nurse insisted that it be given as ordered. Very shortly thereafter he experienced the signs and symptoms of overdosage of epinephrin. He stated that it felt as though there was a powerful explosive force inside attempting to blow off the top of his head. He became extremely nervous and said that his heart was beating at an excessive rate. He was so profoundly affected by this excessive dose of salt solution, that the nurse feared the dyspnea might become worse. She, therefore, suggested that he take another small injection, this was to have been epinephrin. He refused this and stated that he would rather wheeze than take another injection at this time. In a few minutes he was asleep and practically relieved of dyspnea.

At 3:00 A.M. he asked for another injection and was given 5 minims of salt solution with relief of symptoms. At 6:30 A.M. he asked for another hypodermic and was given 8 minims of epinephrin without first trying to obtain relief with salt solution. When I visited him that morning he completely misled me by his description of the toxic reaction. Later when I consulted his chart I learned that he had not received active drug. In this first twenty-four-hour period after salt solution was started he received but 44 minims of epinephrin. This figure seems quite significant in comparison with the amounts previously used. The next night he was given an injection of pantopon, 22 mg. This drug had been used before but would not act as a substitute for epinephrin. For the next three twenty-four-hour periods he required 24, 31, and 16 minims respectively. His private night nurse was then dismissed, following which the amount of epinephrin used increased a little.

He was discharged a week later as he was having practically no asthma and his general condition was much improved. His wife who returned from the East the second day he was in the hospital, helped to continue the psychic therapy after his return home. She reported several weeks later that the salt solution was still quite effective. Recently an acquaintance reported that the patient was so much improved that he looked and acted like an entirely different person.

The psychic factors in this case are too involved to justify a detailed discussion of them. A few additional points may be of interest. He is married to a woman over thirty years his junior—a possible reason for misstating his age. At the time of his marriage about eight years ago he was a successful business man. He was then in a position to command, and undoubtedly did so, immediate response from his subordinates. Now he commands similar attention whenever he has an attack of asthma. He and his two unmarried sisters form an unusual mutual-admiration triad. The sisters openly express their feeling that the patient's wife has stolen the love of their brother, and that God never made a finer man than he. On the other hand the patient intimates that his wife is not sufficiently appreciative of the virtues of his sisters.

On one occasion when he "staged," I use the word advisedly, an attack, just before the four of them were to sit down to supper, the sisters became hysterical as a result of his violent wheezing. They refused to eat and returned to their home. His wife gave him several injections of epinephrin and when he was relieved she proceeded to eat supper. The next day his sisters emphasized to him how tender-hearted they were and how his suffering had completely abolished their appetite.

In repeating this to his wife he intimated that anyone who could eat supper when he had been so ill was very heartless. On the few occasions that his wife attempted to visit friends in the afternoon he would invariably have an attack about the time she was ready to leave. Then he would insist that she could not leave him in his present serious condition. Epinephrin would be resorted to and then he might be free for the rest of the day.

The critical period from 11:00 P.M. to 1:00 A.M. previously mentioned was investigated. While in the hospital his nurse observed that he would awaken during this period without having dyspnea but shortly after he would begin to wheeze and would request a hypodermic.

This habit of awakening at this period did not seem to be dependent upon the type of weather. Ultimately it was learned that at home he would, at about the same hours, call his wife from an adjoining room and request an injection. If she started to go back to bed after he was more comfortable he would insist that she should read to him. Frequently she would sit beside his bed and read to him from midnight until two or three o'clock in the morning. These findings seem to make clear that a psychic mechanism was, in a large measure, responsible for his situation. Possibly a similar mechanism was operating in Case 1 when her attacks began just before the departure of her relatives.

CASE 3.—Miss C., white female, aged twenty-five years. Ancestry is a mixture of French, English, and Irish. Occupation, nurse. She was admitted to the Los Angeles General Hospital a few days before I saw her. On admission she was considered critically ill and she was given a special nurse. The patient stated that she had been taking large amounts of epinephrin for the past two weeks but that it gave her practically no relief. Because of this the drug had not been administered previous to the time I saw her. She had, however, received an enormous amount of supportive and stimulative treatment.

At my first visit she was connected up to rather an elaborate and impressive arrangement of apparatus used to give her oxygen. A tube from this apparatus was kept in her nose by adhesive tape and oxygen was frequently supplied. Not wishing to have the patient exert herself even to the extent of answering routine questions I asked her sister to come out to the ward desk and supply the desired information. This produced an unexpected outburst of temper from the patient, and she made it quite plain that she knew her own history and preferred to give it. This demonstration immediately precipitated a severe paroxysm of wheezing. She became cyanotic and the oxygen was turned on.

Her special nurse was instructed to begin, as soon as possible, the use of psychic therapy combined with hypodermics of sterile salt solution, and to use no opiates unless absolutely necessary. At 8:15 P.M. that evening, August 10, she was given 2 minims of epinephrin and 6 minims of salt solution. This gave considerable relief. At 11:00 P.M. she needed another injection, and made a special request that something be used so that the hypodermic would not be so painful. She was given codeine sulphate, 4 mg. and 2 minims of epinephrin. At 2:30 and 3:00 A.M. she received 10 minims of epinephrin. Unfortunately, salt solution was not tried first, but she was having severe dyspnea and so the nurse thought the patient needed active drug.

The day nurse's notes in the chart for August 11 read "had a very good day." There was marked improvement from her condition in the previous twenty-four-hour period. August 12 at 3:30 A.M. she was given 10 minims of epinephrin. For this twenty-four-hour period the notes read "a very good day." At 11:30 P.M. she was given another injection of 10 minims of epinephrin. She was disconnected from the oxygen apparatus within a few hours after psychic therapy was begun, and was up in a wheel chair August 13, less than seventy-two hours after this method of treatment was started.

Her condition improved so markedly in this period that it seems improbable that it could be due to the total of 22 minims of epinephrin she received in twenty-four-hours. It should be remembered that she had been taking large doses of the drug for a week or two previously without evident relief. If this small amount of the active drug was the important factor in her improvement then it supports my contention that epinephrin is often used in excessive doses.

Sensitivity.—Subsequently the patient was tested, in the Allergy Clinic at the Los Angeles General Hospital, with a large series of food, epidermal, pollen, and other allergens, but no reactions were obtained. She had been tested several years previously and was told that she reacted to feathers and orris root.

DISCUSSION

The ancestry of these patients has been given to show that they are not members of any of the races especially noted for emotional instability.

A traumatic shock is not infrequently the actual or suspected cause of the onset of asthma. In Case 1 the history of such an onset was one of the useful points in determining the line of treatment.

These patients are typical of many others that might be quoted, and they demonstrate that the asthmatic may develop the habit of taking large amounts of epinephrin at frequent intervals, even though he claims little relief from the drug. The periodicity of injection is often striking. It is a common experience that relatively small doses will be effective when given by the physician, but larger ones administered by the patient or family will fail to give relief.

The misuse of epinephrin is often due to the faulty instruction given by the doctor who first advises the "self-medication." The pharmacologic axiom that one should adjust dosage so as to obtain the maximum therapeutic effect and the minimum toxic response with a minimum of the drug, seems to be disregarded in many instances. In the chronically ill patient who may require a certain drug over a period of years, it behooves us to titrate the drug against the patient instead of determining dosage by a fixed rule. Many times the initial dose of epinephrin recommended is 10 to 12 or even 15 minims. Some of these cases might be completely relieved by 2 to 4 minims, and perhaps would not need to have this increased for six months or a year. Any marked increase should be only on advice of the physician. The substitution of another drug for a short time and then a return to epinephrin will frequently defer the increase in dosage for another long period.

The earlier writers recognized, and possibly overemphasized the neurotic element in bronchial asthma. In more recent times, this phase of the problem has too frequently been neglected in favor of specific hypersensitive phenomena, which are assumed by some to explain all the signs and symptoms in these patients. The observations reported herein emphasize the need for a thorough study of each individual patient from, not one, but all the known avenues of approach. The history, as in these cases, will frequently yield a more important clue than will an exhaustive series of skin tests. The thesis that psychic factors are responsible in large part for the habitual use of epinephrin is supported by the results obtained with psychic therapy. Even in the presence of a considerable degree of emphysema, as in Case 2, this form of treatment may be substituted for epinephrin.

SUMMARY

1. Many asthmatics use relatively large and frequently repeated doses of epinephrin when self-medication is permitted.

2. Initial dosage of epinephrin should be determined for each patient, and should be the minimum that will give the desired result.

3. The time at which the injections are given may show a periodicity which cannot be correlated with climate or other extrinsic factors nor with severity of the attacks.

4. When the mental faculties are interestingly occupied, the critical periods may be passed without the use of epinephrin or even the development of clinical symptoms.

5. It is suggested that this is a form of drug addiction.

6. The neurotic element is too frequently disregarded and may be more important than specific phenomena. Asthmatic attacks may create a situation that is quite gratifying to the patient.

7. Psychic therapy and the use of a placebo are valuable aids in the control of epinephrin addiction.

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THE ELIMINATION OF DYES BY THE UTERINE MUCOSA*

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IT HAS been known for many years that glands which have an external secretion may also eliminate various substances injected into the blood stream or absorbed through the intestinal tract. The substances, however, are not eliminated in the same proportion by all organs. In fact, some organs may eliminate practically all of one substance and not any of another even though the substances may be related closely chemically. Phenolsulphonephthalein,[1] for instance, is eliminated almost entirely by the kidney. Phenoltetrachlorphthalein[2] on the other hand is eliminated almost entirely by the liver. Neither of these substances is eliminated by the stomach. Ivy and Dawson[3] studying the elimination of dyes by the stomach mucosa found that of 33 dyes used, only 13 were eliminated in the gastric juice. Crandall[4] found that the pancreas eliminated only 10 of 92 dyes used. Neither in the work on the stomach nor in the work on the pancreas could the result be predicted on the basis of the chemical structure of the dye. Recently, Belfield and Rolnick[5] showed that the body of the epididymis of dogs will eliminate methylene blue, mercurochrome, and silver arsphenamine. They also state that the semen and some of the sperm heads of two men given pyridium by mouth were colored with the dye. The results of the various investigators show, then, that there is a selective elimination of the dye by the secretory organs.

Many drugs have been used in the treatment of infections of the female genital tract. Gentian violet, mercurochrome, and acriflavine have been employed more for the general blood stream infection than for local effect. Kolmer[6] believes that while these drugs may check and eventually remove the bacteremia, they are without marked effect on the local infection.

Because of the inaccessibility of the parts, and because local treatment is sometimes detrimental, any nontoxic antiseptic drug which would be eliminated in appreciable concentration or altogether by the genital mucosa would be theoretically of great value in the management of infections of the female reproductive organs.

Although the drugs recommended for the treatment of genital infections are many and varied, we could find no reference to work showing the elimination of such drugs by the uterine mucosa, therefore, to determine if there

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might be a rational basis for systemic chemotherapy of local female genital infections, we undertook the problem of determining whether the uterine mucosa would eliminate antiseptic dyes which were injected into the blood stream.

METHODS

The experiments were performed on dogs. In all cases it was noted whether they were pregnant, nonpregnant, or postpartum. In the first series of experiments, the dogs were being used in the physiologic laboratory and were under ether anesthesia during the period between the injection of the dye and the removal of the uteri. Thirty dogs were used under these conditions. The results were not constant. In some instances, mercurochrome or pyridium would appear in the mucosa and at other times they could not be demonstrated. Because of the discrepancies in the results and because the anesthesia was possibly an abnormal factor, the results of these experiments were discarded.

The results in Table I are from experiments in which the dogs were injected intravenously with the dye, anesthetized four hours later, the uteri removed and examined grossly and microscopically, without additional staining.

The presence of the dye was determined by the appearance of the mucosa after removal of the uterus. In the control animals and in those in which the dye was not eliminated, the uterine mucosa assumed the usual death pallor. In those in which the dye appeared, the mucosa retained the color produced by the dye. The appearance of the uterus "in situ" could not be used as a guide to the presence or absence of the dye, because in some instances, the uterus would be deep purple or bluish and as soon as removed would become pale. In others, it would be highly injected and deep red, and if the dye did not come through, would immediately turn pale after removal.

TABLE I

DYE USED	DOG	PHYSIOLOGIC STATE OF ENDOMETRIUM	RESULT*	REMARKS
Mercurochrome	1	Not pregnant	Positive	
	2	Postpartum	Questionable	Am't too small
	3	Pregnant	Positive	Did not abort
	4	Postpartum	Positive	
	5	Near term	Positive	Delivered
Pyridium	1	Not pregnant	Questionable	Am't too small
	2	Not pregnant	Questionable	Am't too small
	3	Not pregnant	Positive	
	4	Postpartum	Positive	
	5	Early pregnant	Positive	
	6	Not pregnant	Positive	
Gentian Violet	1	Not pregnant	Questionable	Mucosa darker than normal
	2	Not pregnant	Questionable	Mucosa darker than normal
	3	Postpartum	Negative	
Acriflavine	1	Postpartum	Positive	Deep color
	2	Not pregnant	Positive	Deep color
Methylene Blue	1	Not pregnant	Negative	
	2	Not pregnant	Negative	

*The term "positive" means that the mucosa was stained by the dye, and the term "negative" means that the dye was not detected in the mucosa.

The dyes used were methylene blue, gentian violet, acriflavine, mercurochrome and pyridium. In all cases an aqueous solution was used and in no

instance did the amount of the dye injected reach the per kilo tolerance of man, i.e., 5 mg. per kilo of body weight.

The solutions were injected slowly into the saphenous vein, without anesthesia.

RESULTS

The experiments carried out while the dogs were under anesthesia are not included in the table, but they demonstrated that mercurochrome, pyridium, and acriflavine would stain the uterine mucosa. The results on unanesthetized dogs are shown in Table I. The dyes had no objective toxic action on the animals.

SUMMARY

Mercurochrome, pyridium and acriflavine when injected intravenously gave a distinct color to the uterine mucosa. The result with gentian violet was questionable. Methylene blue did not color the mucosa. It was impossible to determine whether the dye was in the uterine secretion, because of its scantiness in the nonpregnant state and because of its discoloration in the postpartum state.

The physiologic state of the endometrium did not influence the elimination of the dye.

DISCUSSION

Acriflavine[7] and pyridium[8] are said to be bactericidal in dilutions as high as 1:200,000. The value of mercurochrome is questioned. Walker[9] does not believe that mercurochrome in concentrations as high as 1:400 in human blood inhibits the development of staphylococci or streptococci. Young[10] claims good clinical results with mercurochrome used intravenously.

We did not determine the actual concentration or the bactericidal value of the dyes in the uterine mucosa, but intend to do so in future experiments. However, if the degree of color may be used as an indication of the amount of dye present, then mercurochrome and acriflavine reach an appreciable concentration in the uterine mucosa.

In addition to the concentration, the length of time the dyes are in contact with the bacteria is an important factor in inhibiting their growth. The time of appearance and disappearance of the dye was not noted in each of our experiments. We know, however, that the dyes which stained the uterine mucosa appeared there within three hours. In three experiments, in which the uteri were removed twenty-four hours after the injection, the dye was still present. Mercurochrome was used in two of these experiments and pyridium in one. This indicates that the dye may possibly increase its value as a local bactericide.

These experiments were carried out on normal organs and the question arises, will the diseased organ eliminate the dye? This can be answered positively only by experiments on the diseased mucosa.

Hirshfeld, Malmgreen and Creavy[11] reported that mercurochrome and acriflavine will penetrate gradually from the blood stream into edematous

tissues, but did not believe the concentration would be sufficient to be bactericidal.

Young[10] reports mercurochrome of value in localized infections of the male genitourinary tract. Belfield and Rolnick[5] state that silver arsphenamine is of great value in some forms of epididymitis.

As shown by Kolmer[6] ethylhydrocuprein and mercurochrome do not under normal conditions pass into the cerebrospinal fluid, but in pneumococcus meningitis traces of the former, and in streptococcus meningitis traces of the latter, may be found in the cerebrospinal fluid when the dyes are injected intravenously.

The work of Young[10] and of Leonard[12] shows that the kidney may eliminate dyes in sufficient concentration to be of value in infections of the urinary tract.

It may be possible therefore to find an antiseptic dye which will be eliminated from the general circulation by the uterine mucosa in sufficient concentration to be of value in treatment of local infections of the female genital tract.

CONCLUSIONS

The uterine mucosa of dogs will be stained by and may possibly eliminate mercurochrome, pyridium, and acriflavine.

It is questionable whether or not gentian violet will stain the uterine mucosa. Methylene blue does not stain the mucosa under the experimental conditions used. These findings suggest that there is a rational basis for the study of systemic chemotherapy of local disease of the female genital tract.

I wish to thank Dr. A. C. Ivy for generous assistance in conducting this work.

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LEUCOCYTOSIS FOLLOWING INTRACRANIAL OPERATIONS*

By Anatole Kolodny, M.D., Ph.D., F.A.C.S., Iowa City, Iowa

ABOUT fifteen years ago de Quervain called attention to the fact that leucocytosis in a pathologic condition of the abdomen is not always a sign of infection; ruptured ectopic pregnancy is associated with a high leucocytosis. Hoessli[1] demonstrated experimentally on animals that intraperitoneal hemorrhage provokes leucocytosis. Other observers emphasized that leucocytosis follows hemorrhage in other serous cavities, such as the pleural and the cranial. In distinction from the so-called "leucocytosis of hemorrhage," i.e., leucocytosis after loss of blood, when the maximal height of the leucocytosis is reached only after several days and is of a slight degree, leucocytosis after hemorrhage into a serous cavity is much more pronounced and follows immediately after the hemorrhage.

One of the most comprehensive contributions to this question is by Wright and Livingston.[2] In discussing leucocytosis associated with intradural hemorrhage, these authors draw their conclusions from findings in patients who at the postmortem examination showed basal fractures of the skull or an old subdural blood clot and from attempts at basal fractures of the skull in dogs. The difficulties of conducting similar experiments and, therefore, their questionable reliability is obvious.

In the following I present the results of a study of the white cell count in a series of eighteen operations on the brain for various noninfectious pathologic conditions, mostly tumors. In all these cases the leucocytes were counted immediately before operation, and then soon after the operation, the first postoperative count having been made an average of four hours after the completion of the operation. Following this the leucocytes were counted daily for eight days. In twelve of these eighteen cases the operation was on the cerebrum and in six on the cerebellum. In two cases one of the lateral ventricles was opened during the operative procedure. In all instances the operation proceeded intradurally.

Leucocytosis followed the operation in all the cases. The average increase of the number of leucocytes soon after the operation was 210 per cent of the preoperative number. On the first postoperative day this excess of leucocytes over their preoperative number was 144 per cent, on the second postoperative day 96 per cent, on the third 70 per cent, and on the fourth postoperative day 30 per cent. The number of leucocytes reached the normal limits on the fifth or sixth postoperative day.

The leucocytosis in the two cases in which the lateral ventricle was opened was considerably higher than in the other sixteen cases and persisted longer. On the operative day this excess of leucocytes over the normal was

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300 per cent, on the first postoperative day it was 290 per cent, on the second day 200 per cent, on the third day 100 per cent, on the fourth 60 per cent, and on the fifth day 40 per cent. The normal limits were reached on the seventh day (Fig. 1).

A similar study of the leucocytes was made in six other cases in which the dura was reached but not opened during the operation. These cases showed a slight increase in the number of leucocytes, never above 25 per cent over the normal. This leucocytosis reached the normal limits within thirty-six hours after the operation.

Along with an increase in number of the leucocytes the latter showed a marked shift toward the polymorphonuclear variety. At the same time, the lymphocytes and large mononuclears dropped in number, the former far more than the latter. This is illustrated in Fig. 2. This fact is of interest since

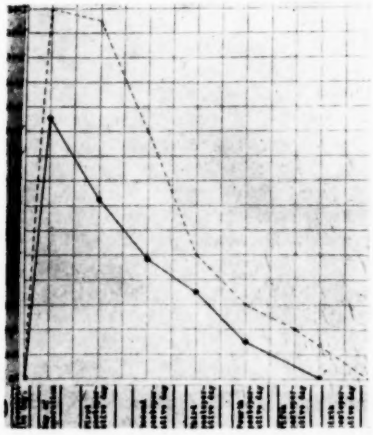

Fig. 1.

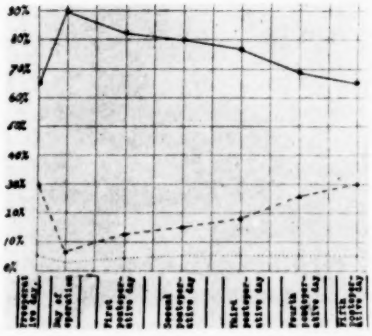

Fig. 2.

Fig. 1.—The average increase of leucocytes over the preoperative number is expressed in per cents of the latter. The heavy line shows the average for all eighteen cases; the broken line shows the average for the two cases in which the lateral ventricle was opened during the operation.

Fig. 2.—This illustrates the differential grouping of the leucocytes prior to and following the operation. The heavy line shows the polymorphonuclears; the broken line shows the lymphocytes and the dotted line the large mononuclears.

it is usually considered that a shift of the differential picture of the leucocytes toward the polymorphonuclears is an indication of an infectious process.

In evaluating the findings of this study the question arises as to the rôle which anesthesia played in the provocation of the leucocytosis. The influence of anesthesia on the leucocytes has been commented upon by a number of observers. It is thought that ether anesthesia (the anesthesia used in most cases of this study) causes a leucocytosis. Judging from the findings of several observers this ether leucocytosis is never over 70 per cent of the preoperative white cell count. In the six cases in which the dura was not opened during the operation the leucocytosis was never above 25 per cent although all of these cases were operated upon under ether anesthesia.

CONCLUSIONS

Craniotomies associated with intradural hemorrhage provoke a high leucocytosis. The degree of leucocytic increase is not proportionate to the amount of bleeding but depends upon whether or not the bleeding occurred intradurally or extradurally. When bleeding takes place into a ventricle, the leucocytosis is higher and of longer duration. The leucocytosis seen in craniotomies without opening of the dura is slight, and the leucocytes return to normal in about thirty-six hours after the operation. To depend upon the differential count for a distinction between an infectious and a noninfectious leucocytosis in intracranial operations is erroneous. Postoperative leucocytosis, when the dura is opened or the ventricle entered, usually shows a differential shift toward the polymorphonuclears.

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EFFECT OF SUBCUTANEOUSLY INJECTED EPINEPHRIN IN NORMAL HUMAN SUBJECTS*

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THE conditions under which subcutaneously injected epinephrin gives a pressor effect in the dog, have been studied by Luckhardt and Koppányi,[1] who found that when the site of injection had been massaged there was a rise in blood pressure of from 15 to 180 mm. Hg following each massage. They also showed that in the dog, at least, epinephrin injected underneath the skin remains there for a long time. In spite of numerous pressor effects obtained as a result of the massage of the epinephrinized area, a saline solution extract made nineteen and one-half hours following the injection of 3 c.c. of epinephrin gave, when injected intravenously, a very marked pressor effect. On the basis of these experiments Lilienthal[2] used this method in the treatment of asthmatic breathing and of surgical shock in man and obtained pressor responses, upon massaging the injected area, for nearly forty-eight hours following the injection of 0.3 c.c. of epinephrin 1:1000. The blood pressure elevations that he obtained were comparatively slight (from 7 to 15 mm. Hg), but he noted very definite therapeutic effects of the drug. Balyeat[3] used the method of massaging the injected areas in various allergic diseases (bronchial asthma, asthma complicated with hypertension, urticaria, hay fever) and reported very encouraging results. Peshkin[4] stated that in children with uncomplicated asthma the massage of the epinephrinized areas did not relieve the

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asthmatic breathing. Dr. A. C. Silverman of the Department of Pediatrics of Syracuse University, on the other hand informs me that in three children (from three to five years old) with uncomplicated asthma (asthmatic bronchitis) the hypodermic injection of 1 c.c. of epinephrin 1:2600 and subsequent massages of the site of injection repeatedly relieved the asthmatic attacks. He noted, however, that children desperately object to massage of the injected areas.

There is no doubt that ephedrine is also a very valuable drug in the treatment of allergic diseases and perhaps also in shock, but because of the favorable results with epinephrin on the one hand, and because of the greater toxicity and relative uncertainty of the action of ephedrine (Halsey[5]) on the other hand, one feels justified in stating that ephedrine has not supplanted epinephrin.

In view of these facts it seemed desirable to investigate the action of subcutaneously injected epinephrin in normal human subjects. Such a study might easily establish a connecting link between the animal experiments and the therapeutic application of the drug.

Six young, healthy, medical students were subjected to subcutaneous epinephrin injections (0.4 c.c. of a 1:1000 solution). Before injection the normal systolic and diastolic blood pressures and the heart and respiratory rates were determined. From 10 to 15 minutes following the injection of epinephrin there was a rise in systolic blood pressure of 30 to 50 mm. Hg without massage. Whereas there was a marked rise in systolic blood pressure, the diastolic pressure generally fell (from 5 to 30 mm. Hg). The pulse pressure thus increased, and as a rule the heart rate became somewhat slower. Thus vagus beats were unmistakably in evidence. The respiratory rate did not change noticeably.

The injected area was blanched, the face pale, there was a slight muscular twitching in the arms and legs, and a thumping, palpitating heart was evident (vagus beats). The subjects felt headache, throbbing in the head and ears, some nausea, the mouth was dry, and there was in some cases subjective sensation of warmth, in others the hands and feet felt cold. Nervousness and general feeling of anxiety were evident in most subjects.

The injected areas were massaged from fifteen minutes to forty-eight hours following injections. In the course of the forty-eight hours the area was massaged about 25 times. On the whole, all the symptoms which appeared following epinephrin injections without massage, could be duplicated by massage of the injected area. They were, however, much less marked, the blood pressure rises, e.g., being seldom more than from 7 to 15 mm. Hg (in agreement with Lilienthal). The rise was practically the same when one massaged the area twenty minutes or twenty hours following the injection. No definite blood pressure elevations upon massage were obtained after forty-eight hours following the injection. In several cases, the depot was depleted thirty to forty hours following the first massage.

If we compare these results with those obtained in the dog, it seems evident that there is a hemodynamic effect following the hypodermic injection of epinephrin in man even without massage, whereas no such effects could be

produced in the dog. Apparently in man epinephrin is more readily absorbed from the subcutaneous tissues. These results are in close agreement with those published by Clough[6] and by Lyon.[7, 8] These authors reported definite initial rises of the blood pressure (without massage) in man, admitting wide individual variations so far as the magnitude of the response is concerned. We can follow Lyon in emphasizing the fact that epinephrin when given subcutaneously is usually rapidly absorbed, but we have to add that a certain amount of epinephrin is retained and forms a depot beneath the skin. This epinephrin depot yields results for about twenty-four to forty hours following injection.

It is scarcely necessary to state that our subjects did not suffer from any thyroid disturbances, and yet they showed blood pressure elevations following epinephrin injections with or without massage. This fact suggests that the Goetsch[9] test is uncertain as a diagnostic procedure, at least so far as the blood pressure rise is concerned.

It is my pleasant duty to thank all my students who aided me in carrying out this investigation.

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LABORATORY METHODS

SOME CHEMICAL ANALYSES OF MEDIUM CULTURED IN LARGE AMOUNTS AND IN SMALL AMOUNTS*

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WHEN chemical analyses are to be made on a medium on which bacteria are growing, a large amount of medium is usually necessary. The medium may be held in a large container from which samples are removed, or the medium may be divided into small amounts sufficient for an analysis, and the separate amounts given the same inoculation and treatment. Having used small samples kept separately for some chemical analyses of milk cultured with bacteria,[1] we thought it would be interesting to find out if there was any significant difference in the results of chemical analyses when the medium was cultured in small samples, or when the medium was kept in one larger container. There are many articles published giving chemical analyses of media kept in either of those ways, but we have not seen any article that gives chemical results from one method compared with results from the other method.

For comparative work, it was advisable to choose a medium that could be easily measured by pipette for the analyses, so a broth medium was used. A dehydrated dextrose broth was dissolved in distilled water, in the proportion of 13 gm. powder to 1 liter water. For the small samples, the broth was put into culture tubes, one inch by eight inches. For the large amounts, a Squibb's pear-shaped, separatory funnel of one liter size was used.

The details of handling the medium with the different bacteria varied slightly, but care was taken that the conditions were the same for each bacterium. The separatory funnels and culture tubes were plugged with non-absorbent cotton and sterilized in the autoclave at 15 pounds pressure for twenty minutes on three days. The dehydrated dextrose broth was dissolved in one large container. Eleven to twelve hundred c.c. of medium were put into each separatory funnel and these were weighed. About 75 c.c. medium were put into each culture tube and then enough more medium was added to bring medium, tube, and holder to a definite weight so that the final weight of all tubes with the holder would be the same. The separatory funnels and culture tubes with the medium were sterilized once in the autoclave and then incubated. All samples were incubated at 37° C. The bacteria to be used for inoculation were grown for two days on the same dextrose broth as that used for the analyses. The bacteria used were B. acidi lactici, Streptococcus lacticus, and a gram-positive, spore-bearing bacillus resembling in many of its

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TABLE I

STREPTOCOCCUS LACTICUS

	HYDROGEN-ION CONCENTRATION (P_H)						TITRATABLE ACIDITY (C.C. 0.1 N NaOH PER 10 C.C. MEDIA)						AMMONIA (C.C. 0.02 N H_2SO_4 PER 5 C.C. MEDIA)					
	LARGE AMOUNTS			SMALL AMOUNTS			LARGE AMOUNTS			SMALL AMOUNTS			LARGE AMOUNTS			SMALL AMOUNTS		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Control	6.7*	6.8	6.8	6.8	6.8	6.7	0.28*	0.31	0.30	0.30	0.29	0.30						
Control incubated	6.7	6.8	6.8	6.8	6.8	6.8	0.31	0.31	0.31	0.30	0.31	0.30	-0.01*	0.10	0.05	0.04	-0.05	0.04
1 Day	4.2	4.2	4.2	4.2	4.2	4.2	1.53	1.53	1.55	1.57	1.56	1.57	0.55	0.56	0.41	0.55	0.45	0.51
2 Days	3.2	3.2	3.2	3.2	3.2	3.2	1.83	1.82	1.78	1.90	1.89	1.87						
3 Days	3.2	3.2	3.2	3.2	3.2	3.2	1.90	1.88	1.80	1.97	1.97	1.98	0.61	0.58	0.54	0.56	0.52	0.46
4 Days	3.2	3.2	3.2	3.2	3.2	3.2	1.93	1.9	1.82	1.91	1.93	1.96						
7 Days	3.2	3.2	3.2	3.2	3.2	3.2	1.91	1.83	1.78	1.89	1.89	1.91	0.63	0.56	0.59	0.6	0.49	0.48
31 Days	3.2	3.2	3.2	3.2	3.2	3.2	1.90	1.79	1.75	1.97	2.01	2.00	0.62	0.48	0.48	0.57	0.46	0.42

*Each result in the table is the average of three determinations.

cultural reactions Bacillus flexus. To inoculate with B. acidi lactici and Streptococcus lacticus, 1 gm. of inoculated broth was added to each tube, and 15 gm. of inoculated broth were added to each separatory funnel. For the spore-bearing bacillus, 1 drop inoculated broth was added to each tube and 15 drops of inoculated broth were added to each separatory funnel.

To obtain samples for analysis from the separatory funnels, sterilized water was added, to make up for the loss in evaporation, until the weight was the same as that of the last weighing, the medium was shaken, and an approximate volume of medium let out through the stopcock into another container. Water was added to the medium in the culture tubes until the weight was the same as that before sterilization.[2] Medium cultured in three separatory funnels and in three tubes was analyzed at one time, and each analysis was done in triplicate.

The chemical analyses made were hydrogen-ion concentration, acidity, and ammonia. The hydrogen-ion concentration was determined colorimetrically. Standards were prepared using 0.2 M disodium phosphate and 0.1 M citric acid according to the table given by McIlvaine.[3] Mercurous chloride was used to prevent mold growth[4] in the standards, and they were kept in a dark cupboard when not in use. The indicators were chosen from those given by Kolthoff;[5] and as he suggests, made with alcohol when necessary, and water. The acidity was determined by titrating 10 c.c. medium with 0.1 N NaOH using phenolphthalein as indicator. The method used for the determination of ammonia is that of Van Slyke and Cullen.[6, 7] The ammonia from 5 c.c. medium was aspirated into 25 c.c. 0.02 N H_2SO_4 and the excess acid titrated with 0.02 N NaOH.

The media on which Streptococcus lacticus and the spore-bearing bacillus had been grown, when examined bacteriologically at the end of the series of chemical analyses, showed that no contamination had occurred.

The table for Streptococcus lacticus is given as typical. The figures for the medium cultured in large amounts agree closely. Also the figures for the medium cultured in small amounts are much alike. This agreement indicates that the growths of the bacteria were sufficiently uniform to show no marked differences in their metabolism products when estimated chemically. As the values found for the medium cultured in large amounts agree with those for the medium cultured in small amounts, it would seem to make little difference, as far as chemical analyses are concerned, whether the medium is cultured in one large amount or in separate small amounts.

With these estimations, there might be included some work done on milk that was coagulated by a bacterium during growth. This medium is not as easily measured as broth, so that the results might show more variation than those for broth. Sugar estimations only were done in the milk series. The method used was taken from Leach, *Food Inspection and Analysis*.[8] The milk was measured by pipette, and the protein precipitated with Fehling's copper sulphate solution and 0.5 N NaOH according to Soxhlet's method (Leach, p. 150). Water was added and the mixture filtered. The filtrate was used with Fehling's solution as given in the Defren-O'Sullivan method (Leach, p. 594).

The precipitate of cuprous oxide was washed with water, alcohol and ether, dried in the oven, and weighed as cuprous oxide.

All these analyses were done on milk kept in small amounts. About 50 c.c. fat-free milk were put into culture tubes stoppered with nonabsorbent cotton. The volume was marked on the tube and water was added to the mark before the milk was taken for analysis. The first series was done to see how close the agreement would be among milk samples measured from the same tube. Three amounts of milk from one tube were measured into separate containers, the protein precipitated, and the mixture filtered. From each filtrate, three separate amounts of filtrate were taken for the cuprous oxide precipitation, the precipitates filtered and weighed separately and the triplicate weights averaged for the results shown in the table. In the second series, milk from different tubes was taken to see if the results would vary more than the results from different samples of milk measured from the same tube. One sample of

TABLE II

STREPTOCOCCUS LACTICUS

	SUGAR IN GM. Cu_2O		
	SAMPLES FROM SAME TUBE		
	A	B	C
Control	0.1737*	0.1741	0.1746
Control	0.1730	0.1736	0.1773
Incubated 3 days	0.1415	0.1459	0.1445
Incubated 4 days	0.1421	0.1419	0.1465
Incubated 5 days	0.1394	0.1374	0.1432
Incubated 6 days	0.1422	0.1319	0.1362
Incubated 12 days	0.1408	0.1371	0.1393
Incubated 13 days	0.1397	0.1420	0.1387
Incubated 16 days	0.1329	0.1328	0.1414
Incubated 17 days	0.1342	0.1356	0.1413
Control. Incubated 21 days. Not made to original volume	0.1893	0.1899	0.1898
Control. Incubated 22 days. Not made to original volume	0.1877	0.187	0.1944
Control. Incubated 23 days. Not made to original volume	0.1887	0.1883	0.193

*Each result in the table is the average of three determinations.

TABLE III

STREPTOCOCCUS LACTICUS

	SUGAR IN GM. Cu_2O		
	SAMPLES FROM DIFFERENT TUBES		
	A	B	C
Control	0.1825*	0.1858	0.1874
Control	0.1774	0.1842	0.1835
Control	0.1809	0.1852	0.1793
Incubated ½ day	0.1565	0.1518	0.1529
Incubated 2 days	0.1356	0.1461	0.1436
Incubated 5 days	0.1450	0.1367	0.1446
Incubated 6 days	0.1529	0.1531	0.1413
Incubated 15 days	0.1496	0.1494	0.1543
Incubated 16 days	0.1467	0.1488	0.1463
Incubated 19 days	0.1488	0.1399	0.1462
Incubated 20 days	0.1366	0.1422	0.1460
Control. Incubated 27 days	0.1748	0.1755	0.1749
Control. Incubated 28 days	0.1710	0.1726	0.1709
Control. Incubated 29 days	0.1729	0.1779	0.1744

*Each result in the table is the average of three determinations.

milk was measured from three different tubes, the protein precipitated, and the mixture filtered. From each filtrate, precipitations of cuprous oxide were made in triplicate, and the three weights averaged to give the values as reported.

The milk reported in Table II was inoculated with 1 c.c. Streptococcus lacticus culture in each tube; and the milk reported in Table III was inoculated with ½ c.c. Streptococcus lacticus culture in each tube. More work was done but as the other tables show similar results, only these two are given. As will be seen from the tables, the results of the analyses of sugar estimations done by this method when the milk is cultured in separate tubes, do not vary significantly from the results of more than one estimation done on milk from the same container.

CONCLUSIONS

From the results of these estimations, we can conclude that for hydrogen-ion concentration, titratable acidity, and ammonia estimations in broth, it makes little difference whether the medium is cultured in large amount and samples removed at various times, or whether the medium is cultured in separate containers for each set of estimations. We may also conclude that estimations of sugar in coagulated milk kept in separate amounts under the same conditions do not show any more variation than those in different samples taken from the same container.

I wish to acknowledge my indebtedness to Dr. G. A. Davis, who supplied the bacteria, and made the bacteriologic examinations.

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A NEW METHOD FOR COUNTING BLOOD PLATELETS IN UNDILUTED CITRATED PLASMA*

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THE blood platelet count and its variations occupy an important position in hematology. The size and extravascular fragility of the platelets, however, have impeded the progress of knowledge of their genesis, morphology, function, and number.

Numerous methods for counting blood platelets have been published, but the variations of the "normal number" and the great fluctuation within short intervals indicate that none of the methods has been able to surmount the difficulties of exact enumeration, or to eliminate technical errors. In attempts to meet the difficulties, few investigators have realized how many and varied are the factors that may serve as sources of error. Obtaining the blood (by skin incision or vein puncture) and actual counting (direct or indirect), usually in a counting chamber, do not admit of many variations. The modifications, therefore, of the various methods have chiefly dealt with the diluting fluid.

A great advance in the technic of counting blood platelets was made by the introduction of Oluf Thomsen's[1] macro-method in 1919. The new and essential part of this method was the segregation of the blood platelets, advantage being taken of the difference of rate of sedimentation of the platelets and the other blood cells in stabilized blood.

In Thomsen's macro-method 4.5 c.c. of blood, obtained by vein puncture, was dropped into a graduated test tube containing 0.5 c.c. of a 10 per cent (or 3 per cent) solution of sodium citrate. After shaking, the sample was left for an hour or more for sedimentation. A small amount of the plasma from the surface of the sample (containing platelets) was diluted with physiologic saline in a convenient proportion, usually 1 to 20. The direct counting of the blood platelets was carried out in a Thoma-Zeiss counting chamber after fifteen to thirty minutes. The number of blood platelets in the plasma per c.mm. was calculated from the simultaneous determination of the volume of red blood cells.

This method was quickly introduced in Denmark. It is the chief method in hematology manuals[2, 3] and is still used in many hospitals. It was used in several clinical investigations by Gram,[4] Rud,[5] Kühnel;[6] in animal experimentation by Fabricius-Möller,[7] Petri;[8] and both clinically and experimentally by Wittkower.[9]

Unfortunately the method has certain disadvantages limiting its usefulness. As the blood is obtained by vein puncture, it is difficult to do the count

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on small children, and it imposes certain limitations on serial examinations. Also, because of the dilution of the serum before filling the counting chamber, the shape, size, and number of the blood platelets are altered, an important defect in most methods. The method is also useless for serial examination in most laboratory animals, except when severe anemia is desired. The error of the method calculated by Meulengracht and Gram[2] was 5 per cent. In ten counts, I found it to be 21.9 per cent with diluted plasma (number of platelets 678,400), and 10.4 per cent using undiluted plasma (number of platelets 437,000).

To remedy some of these deficiencies, Oluf Thomsen devised a micro-method in 1923[1] based on the same principle. From a skin incision 0.09 c.c. of blood was obtained by pipette and mixed in a small tube or on a glass slide with the same proportion of sodium citrate as in the macro-method. The mixture was drawn into a capillary tube and allowed to stand for sedimentation and the counting done in a Thoma-Zeiss chamber ($^1/_{10}$ mm. high) on the undiluted or the diluted plasma.

This method was never used in the clinic, and to a small extent only in animal experimentation by Anderson[10] on rats and Petri[8] on rabbits. The percentage of error was calculated by Als[11] as 8 to 10 per cent and by Petri[8] as 20.3 per cent.

To make Oluf Thomsen's principle suitable for serial investigations in laboratory animals it was necessary to determine the percentage of error accurately, as well as the influence of more or less radical changes in the technic on the blood platelets in the blood sample. The results obtained in testing individual features of Oluf Thomsen's method are applicable to most methods for counting blood platelets previously reported.

Thus, when counting platelets, *it is necessary to distinguish the samples obtained by skin incision from those taken by vein puncture.* An admixture of fluid from the tissues can never be avoided in skin incision, and this enhances the tendency of the platelets to shrink. This is evident to a certain degree while the blood is flowing and while it is standing, but especially when the plasma is diluted. The rate of sedimentation of the blood platelets is also diminished, and adhesions will occur between the blood platelets and the erythrocytes. In samples obtained from a vein, however, the size, shape, and number of the platelets remain unchanged for a long time.

In the process of dilution preceding the counting, changes in size and shape take place in the platelets, especially in the blood obtained by skin incision. The average error therefore is considerably larger in the diluted than in the undiluted plasma.

From a thorough investigation of different salt solutions, fixation fluids, acids, etc., it appears that none of the dilution fluids used wholly prevent the shrinkage of the blood platelets. It seems as if the platelets were unable to withstand any appreciable change of the medium in which they are normally suspended. As regards the stabilization medium, sodium citrate seems to be the most serviceable.

If blood platelets, therefore, are counted without considering the points mentioned above, the reliability of the count suffers. In Oluf Thomsen's

macro-method the plasma was diluted, in the micro-method the blood was obtained by incision. Thus both methods have inherent faults. To count blood platelets accurately one must, (1) simplify method, (2) avoid admixture of tissue juices, (3) stabilize blood properly, (4) avoid dilution, and (5) separate the blood platelets as quickly as possible from the white and red blood cells.

To exclude the most obvious sources of error a new technic, based on the method given by Oluf Thomsen, has been devised, subject to minor modifications. This method is applicable to both man and experimental animals, and is especially suitable for serial examinations (one or more times a day) in rabbits. It is as follows:

1. Draw 0.45 c.c. of blood from the auricular vein into a 1 c.c. syringe containing 0.05 c.c. of 10 per cent sodium citrate. For the puncture, a 2 cm. long, medium-sized needle, cut straight transversely, is used. Both syringe and needle are impregnated with liquid paraffin, and the excess squirted out before the sodium citrate is drawn into the syringe.

2. Transfer blood from syringe into small paraffined test tube, close with rubber stopper, shake gently a few times, then centrifuge for one minute. In a centrifuge with 5000 revolutions a minute, one minute is adequate for a ½ c.c. blood sample. The time was divided as follows: ¼ minute half speed, ¼ minute full speed, ¼ minute half speed, and ¼ minute for stopping. Centrifuging takes the place of the sedimentation by standing in Oluf Thomsen's method. It is important, however, that in centrifuging, the procedure be carried out in the same manner every time. Any centrifuge can be used; it is only necessary once for all to determine the exact time necessary. Because of the great difference in the rate of sedimentation between the erythrocytes and the blood platelets, the erythrocytes will be thrown down from the upper ⅓ or ½ of the sample before the original content of blood platelets has been changed.

3. Remove a small amount of the blood platelet containing citrated plasma with a fine capillary, place it directly on a $\frac{1}{20}$ mm. high Thoma-Zeiss counting chamber, cover tightly with a cover glass. In order to avoid air bubbles, the cover glass is held obliquely when put in place. Avoid contaminating the sample with liquid paraffin which covers the plasma after centrifuging.

4. Allow to stand fifteen minutes for sedimentation. After this time the platelets will be found to be refractive, sharply outlined, uniform, slightly oval, discoid corpuscles of equal size, evenly distributed in the counting chamber.

5. The platelets are counted in 8 small squares. The sum denotes the number of blood platelets per 1 c.mm. of plasma. It is superfluous and unnecessarily complicated to recalculate the blood platelet figures found in the citrated plasma per c.mm. of blood.

In handling rabbits it is convenient to confine them to an animal box with the head protruding through a circular opening. The hairs are removed from the ear, the skin is cleaned, and the vessels made prominent by rubbing with

toluol. To avoid loss of blood the needle is introduced subcutaneously for about ½ cm. before the vein is punctured. At the same time the needle is fixed with a clip, the prongs of which are attached to the ear above and below the puncture. The needle is removed quickly after the blood has been obtained, and the puncture is at once closed with the clip. Blood may also be obtained by heart puncture, but vein puncture is by far the easiest and safest method. In a medium-sized rabbit the loss of 1 c.c. of blood daily for ten to twelve days will not bring about any noteworthy change in the erythrocyte and platelet count. In smaller animals, like guinea pigs, rats, and mice, the blood must be obtained by heart puncture, and it is only possible to make serial investigations every second or third day on account of the danger of a hemorrhagic anemia.

In man the blood is obtained by the puncture of a vein, in a 5 c.c. syringe, or directly in a test tube, stabilizing the sample with 10 per cent sodium citrate in the proportion of 1 to 10. In small children, where vein puncture may be impracticable, one may be forced to obtain blood by the earlier, less reliable methods, i.e., a freely bleeding prick or incision.

The accuracy of the "centrifuging method" without any dilution of the blood has been determined by making six ten-counts. The percentage of error, when the blood had been taken by vein puncture, was 7.3 per cent (minimum 7.1 per cent) per 760,000 platelets and when blood had been taken by skin incision 10.5 per cent (minimum 9.4 per cent) per 380,000 blood platelets.

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A RAPID METHOD FOR THE PREPARATION OF FECAL DIGESTS SUITABLE FOR USE IN NITROGEN AND MINERAL ANALYSES*

By Genevieve Stearns, Ph.D., Iowa City, Iowa

ANALYSES of feces are usually extremely tedious. The use of fecal digests which may be pipetted† greatly increases the speed of an analysis, without decreasing its accuracy. A method for the preparation of such digests has been in use in this laboratory for over a year, and has proved to be efficient. No claim is made for originality in the method; it is merely a time-saving combination of methods that has been tested and found useful.

The feces are collected in the usual manner.[1] If only nitrogen and mineral analyses are wanted, the fecal specimens may be put directly into a 2 or 3 liter wide-mouthed Erlenmeyer flask containing about 600 to 800 c.c. of 10 per cent sulphuric acid. The flask is kept in a refrigerator, the successive specimens being added until collection is complete. The whole material is then brought to a boil in the hood, adding more acid if necessary, so that the mixture will be thin enough to pour well and pipette readily. The boiling is continued for from five to ten minutes, being careful to avoid too vigorous heating, or foaming will occur. As soon as the solid material is well broken up, and the mixture appears of even consistency, the contents of the flask are cooled to room temperature, and transferred to a 1 or 2 liter volumetric flask, pouring the material through a sieve (an ordinary small sieve, such as is used for a tea strainer, 15 to 20 mesh, is suitable). If the strainer is placed in a funnel, which, in turn, is inserted in the neck of the flask, the transfer is simple. The solid material collecting on the sieve can be forced through by gentle pressure with a spatula, so that the only residue left on the sieve, after washing is complete, consists of seeds and like material, the weight, in most diets, being so small that it may be disregarded. If the diet contains an unusual amount of undigestible or difficultly digestible material, so that the residue which does not pass through the sieve is too large to be disregarded, the remaining material may be transferred to a Kjeldahl flask and heated with concentrated sulphuric acid until solution takes place. It is not necessary to carry the digestion on to completion, just to the point where solution is com-

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†A sulphuric acid digest of feces used by Dr. O. H. Gaebler of the Department of Biochemistry, State University of Iowa, for nitrogen determination, was described to us in a personal communication.

plete. This second digest is cooled, diluted somewhat, cooled under running water and added to the original material. The boiling flasks, sieve, and funnel are washed well with distilled water and the washings added to the original mixture, which is then made up to volume and mixed well. The suspension thus formed lasts for sufficient time to enable sampling with a pipette. It may be kept at room temperature without deterioration, but must be thoroughly mixed before sampling. This digest will be referred to as "Digest A."

NITROGEN AND CHLORIDE

Digest A is used directly for nitrogen and chloride determinations. The amount necessary for a macro-Kjeldahl will vary somewhat with the dilution of the digest, but we have found that 10 c.c. samples are usually satisfactory. We have used the Kjeldahl-Gunning technic as for nitrogen in urine.

If chlorides are to be determined in feces, certain precautions are necessary in the preliminary boiling in order to guard against loss of hydrochloric acid. The boiling flask is connected to a condenser, and the distillate collected in dilute alkali. Chloride determinations are run on this distillate, and the amount found (usually very small) is added to the total otherwise determined. Ten c.c. samples of Digest A are taken for the chloride determination, using the Wilson and Ball[2] modification of the Van Slyke method for chloride in blood and tissues.[3]

CALCIUM, PHOSPHORUS, AND TOTAL FIXED BASE

Twenty-five c.c. samples of Digest A are placed in 250 c.c. Erlenmeyer flasks, 5 c.c. of concentrated sulphuric acid are added, and the mixture is heated on a hot plate in the hood until most of the water is driven off. The flasks should be shaken occasionally to prevent bumping. After cooling for a minute, concentrated nitric acid is added, 1 to 2 c.c. at a time, heating after each addition until nitric oxide fumes are no longer given off. When all the organic matter is digested, the solution will be clear and colorless or but slightly yellowish. If a crystalline precipitate has formed, it is brought into solution with nitric acid and water, keeping the concentration of nitric acid as low as possible. The solution is cooled, transferred quantitatively to a 100 c.c. volumetric flask, and made up to volume. This solution (Digest B) is used for the determination of calcium, phosphorus, and total fixed base.

Calcium.—A modification of the McCrudden method[4] has been used for the determination of calcium in fecal digests. One hundred c.c. of Digest B is pipetted into a 150 c.c. pyrex beaker, and the solution neutralized with concentrated ammonium hydroxide, using rosalic acid (0.5 per cent in 95 per cent alcohol) as indicator. Concentrated hydrochloric acid is then added until the color just changes to a definite yellow. Ten c.c. of 2.5 per cent oxalic acid are then added, the solution is boiled for about one minute, then 10 c.c. of 20 per cent sodium acetate are run in drop by drop, and the solution allowed to stand several hours or overnight. The liquid after the addition of the acetate should be tan in color; if the yellow color persists, more acetate should be added.

The calcium oxalate is filtered off by suction, using Jena glass filter crucibles, No. 10G/<7. The beaker and filter are washed twice with dilute am-

monium hydroxide solution (20 c.c. of the concentrated reagent per liter); the outside of the crucible is washed off with distilled water, and the crucible returned to the beaker. The precipitate is dissolved in 10 c.c. of approximately normal sulphuric acid (28 c.c. of concentrated sulphuric acid per liter), heated to 90° C. and titrated with 0.02 N permanganate, keeping the temperature between 70 and 90° C., and following the usual technic of permanganate titrations.

Calculation.—

As 1 c.c. of 0.02 N permanganate is equivalent to 0.4 mg. of calcium, the calculation will be as follows:

$$\text{Titration value} \times \frac{\text{normality of } KMnO_4}{0.02} \times 0.4 \times \frac{\text{Total vol. digest B}}{\text{vol. sample of B}} \times \frac{\text{Total vol. digest A}}{\text{vol. sample of A}}$$

This reduces to:

$$\text{Titration value} \times \frac{\text{normality of } KMnO_4}{0.02} \times 0.4 \times \frac{100}{10} \times \frac{\text{Total vol. digest A}}{25}$$

or

$$\text{Titration value} \times \text{normality of } KMnO_4 \times \text{total vol. digest A} \times 8 = \text{Mg. calcium in total stool.}$$

All reagents should be tested for calcium and a blank run if necessary. We have found that many samples of so-called c.p. sodium acetate are contaminated with calcium. By making up the solution in 0.5 per cent ammonium oxalate instead of distilled water, and filtering off the resulting precipitate, most of the calcium salts are removed.

Phosphorus.—The Fiske and Subbarow method[5] for inorganic phosphate in urine may be used with Digest B, if the excess acidity is first neutralized. The sample (4 to 10 c.c. depending on the phosphate content) is placed in a 100 c.c. volumetric flask and diluted to about 60 c.c. with distilled water. After the addition of one drop of phenolphthalein, ammonium hydroxide is added just to a faint pink coloration. The determination is then carried out in the usual manner, the acidity of the reagents destroying the pink color.

Total Fixed Base.—The method of Fiske[6] for the determination of total fixed base in urine may be used with Digest B. The digestion with 4N sulphuric acid may be omitted, all organic matter having been removed in the preparation of the digest.

THE METHOD AS APPLIED TO THE FECES OF INFANTS AND SMALL CHILDREN

When the diet consists largely of milk, the calcium content of the stool is often so high that a heavy crystalline precipitate of calcium sulphate may form in Digest A. The digest is boiled until the precipitate is coarse, so that the supernatant material can readily be decanted from it. The liquid is decanted into a liter volumetric flask, the precipitate is washed well with several successive portions of distilled water and the washings decanted carefully into the original mixture, which is made up to a volume of 1 liter (Digest A). The precipitate is dissolved in dilute nitric acid and made up to a volume of 250 c.c. (Solution C). A mixture of equivalent portions of A and C is then used in the preparation of Digest B; for example, a 100 c.c. portion of the well mixed Digest A is added to 25 c.c. of Solution C and 25 c.c. samples of this mixture are digested with nitric and sulphuric acids, cooled and made up to a volume

of 100 c.c. In calculating, 1250 c.c. is used as the total volume of all determinations run on Digest B. Nitrogen and chloride determinations are run on Digest A in the manner previously described using 1000 c.c. as the total volume.

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A NEW METHOD FOR CALCULATING COLORIMETRIC RESULTS*

By J. Cletus Baier, Baltimore, Md.

THE method consists of setting the unknown solution, instead of the standard. In this way a constant factor will be obtained by which all determinations of the same type can be multiplied. Below is a more detailed account of the method of determining the factor to be used in each case, together with a comparison of the present method.

BLOOD SUGAR: (FOLIN-WU OR BENEDICTS)

Using Standard Containing 0.2 mg. Sugar

Present Method

$$\frac{20 \times 100}{R} = \text{mg. S per 100 c.c. blood.}$$

New Method

Using 0.2 mg. standard

$$\frac{R \times 100}{20} = \text{mg. per 100 c.c. blood.}$$

In the new method

20 represents the setting of the unknown.
R represents the reading of the standard against the unknown set at 20.
100 is the same as in the old method.

It can readily be seen that by the old method the reading of the unknown is subject to constant change, hence each determination is a new calculation. In the new method, since the unknown is set at a definite depth, the same figure is used for every determination, while the top figure representing the reading of the standard against the unknown, changes. Since the figure representing the unknown does remain the same in the new method, the following cancellation may be made, thereby greatly simplifying the calculation.

$$\frac{R}{\cancel{20}_{4}} \times \frac{5}{\cancel{100}_{1}} = \text{mg. Sugar per 100 c.c. blood}$$

Therefore 5 will be the constant factor for blood sugars.

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Example.—With the standard reading 16.4 against the unknown.

$$16.4 \times 5 = 82 \text{ mg. per 100 c.c. blood}$$

Reading of 23.1

$$23.1 \times 5 = 115.5 \text{ mg. per 100 c.c. blood}$$

BLOOD NONPROTEIN NITROGEN (FOLIN-WU METHOD)

Standard contains 0.15 mg. nitrogen.

Present Method.

$$\frac{20 \times 30}{\text{R of unknown against standard}} = \text{mg. NPN per 100 c.c. blood}$$

New Method.

$$\frac{\text{R of standard against unknown} \times \overset{1.5}{\cancel{30}}}{\underset{}{\cancel{20}}} = \text{mg. NPN per 100 c.c.}$$

Since setting the unknown at 20 in this case gives an inconvenient factor, 15 may be used instead.

$$\frac{\text{R} \times \overset{2}{\cancel{30}}}{\cancel{15}} = \text{mg. NPN per 100 c.c. blood. Two is the factor to be used}$$

Example.—A reading of 17.5 for the standard against the unknown would be $17.5 \times 2 = 35$ per nitrogen per 100 c.c. blood.

BLOOD CREATININE (FOLIN-WU)

Present method when using weak standard.

$$\frac{20 \times 1.5}{\text{R of unknown}} = \text{mg. Creatinine per 100 c.c. blood}$$

New Method as in the NPN calculation against the setting of 15 may be used instead of 20.

$$\frac{\text{R of standard} \times \overset{0.1}{\cancel{1.5}}}{\cancel{15}} = \text{mg. Creatinine per 100 c.c. blood. One-tenth is the factor.}$$

Example.—With a reading of 13.3.

$$13.3 \times 0.1 = 1.33 \text{ mg. Creatinine per 100 c.c. blood}$$

BLOOD URIC ACID (BENEDICT OR FOLIN-WU)

Present Calculation.

$$\frac{20 \times 4}{\text{R of unknown}} = \text{mg. per 100 c.c. blood}$$

New Method.

$$\frac{\text{R of Standard against unknown} \times \overset{0.2}{\cancel{4}}}{\cancel{20}} = \text{mg. per 100 c.c. blood. Factor to be used is 0.2.}$$

CONCLUSION

By setting the unknown instead of the Standard in colorimetric determinations a constant factor will be obtained which will save time and trouble in calculations. This method is not only applicable to blood analysis, but to any analysis where the colorimeter is used.

929 S. CLINTON STREET.

A RAPID AND CONVENIENT METHOD FOR SKIN TESTING WITH POLLENS*

By John P. Henry, M.D., Memphis, Tenn.

PRACTICALLY all of the various methods used in performing the "scratch" tests with pollens are satisfactory as far as determining positive or negative reactions is concerned. Some of these methods, however, are more laborious than others and it was a consideration of the time element which led us to adopt the method about to be described.

Two per cent solutions of all the various pollens with which we are concerned in this territory were prepared by extraction in 7 per cent sodium chloride and 46 per cent glycerin, as described by Stier and Hollister.[1]

Fig. 1.

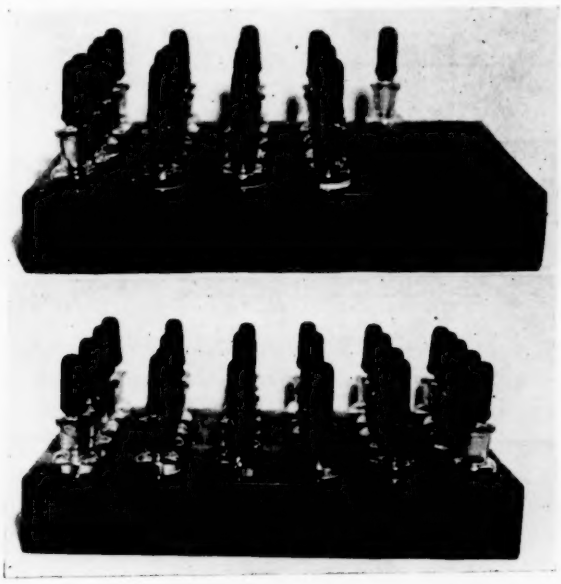


Fig. 2.

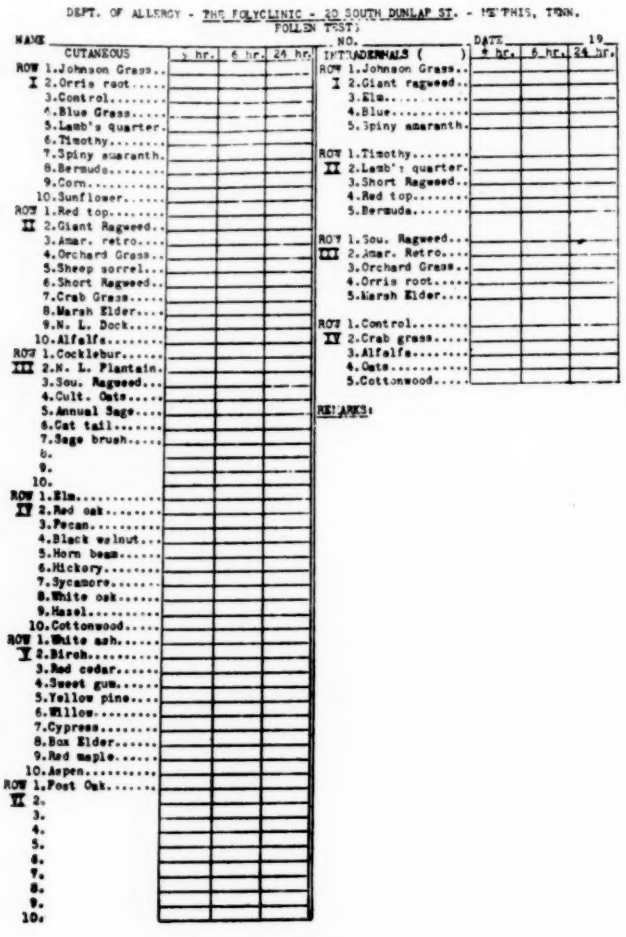
Five c.c. of each solution was transferred to sterile 15 c.c. bottles. Ground glass pipettes with heavy rubber nipples keep these bottles tightly stoppered when not in use. The nipples are so adjusted that it is impossible to draw the pollen solution into the rubber portion. (Fig. 1.)

Two trays were then devised, using heavy boards measuring 11 inches in length, 8 inches in width, and 1½ inches in thickness. This size was adopted to fit our ice box. Thirty holes, six rows of five each, with a diameter of 1⅛ inches were then bored through these boards. Thin boards, 3/16 inch, were then tacked on to act as floors. One tray is mahogany in color and contains

*From the Department of Allergy, The Polyclinic, Memphis, Tenn.

our tree pollens. The other tray is of lighter color and contains the grasses and weeds. (Fig. 2.)

The testing bottles are kept in the trays in a definite order. To save further time, mimeographed sheets are used in recording readings. These contain a list of the pollens exactly as they are arranged in the trays. A space is provided opposite each for recording results. This eliminates all writing other than recording "0" for negatives; +, ++, +++, or ++++ for positives. These sheets are the same size as our history sheets and are filed as a part of the record. (Fig. 3.)


DEPT. OF ALLERGY - THE POLYCLINIC - 20 SOUTH DUNLAP ST. - MEMPHIS, TENN.

POLLEN TEST

NAME _____ NO. _____ DATE _____ 19__

	CUTANEOUS	½ hr.	6 hr.	24 hr.	INTRADERMALS ( )	½ hr.	6 hr.	24 hr.
ROW I	1. Johnson Grass				1. Johnson Grass			
	2. Orris root				2. Giant ragweed			
	3. Control				3. Elm			
	4. Blue Grass				4. Blue			
	5. Lamb's quarter				5. Spiny amaranth			
	6. Timothy							
	7. Spiny amaranth				1. Timothy			
	8. Bermuda				2. Lamb's quarter			
	9. Corn				3. Short Ragweed			
	10. Sunflower				4. Red top			
ROW II	1. Red top				5. Bermuda			
	2. Giant Ragweed							
	3. Amar. retro				1. Sou. Ragweed			
	4. Orchard Grass				2. Amar. Retro			
	5. Sheep sorrel				3. Orchard Grass			
	6. Short Ragweed				4. Orris root			
	7. Crab Grass				5. Marsh Elder			
	8. Marsh Elder							
	9. N. L. Dock				1. Control			
	10. Alfalfa				2. Crab grass			
ROW III	1. Cocklebur				3. Alfalfa			
	2. N. L. Plantain				4. Oats			
	3. Sou. Ragweed				5. Cottonwood			
	4. Cult. Oats							
	5. Annual Sage				REMARKS:			
	6. Cat tail							
	7. Sage brush							
	8.							
	9.							
	10.							
ROW IV	1. Elm							
	2. Red oak							
	3. Pecan							
	4. Black walnut							
	5. Horn beam							
	6. Hickory							
	7. Sycamore							
	8. White oak							
	9. Hazel							
	10. Cottonwood							
ROW V	1. White ash							
	2. Birch							
	3. Red cedar							
	4. Sweet gum							
	5. Yellow pine							
	6. Willow							
	7. Cypress							
	8. Box Elder							
	9. Red maple							
	10. Aspen							
ROW VI	1. Post Oak							
	2.							
	3.							
	4.							
	5.							
	6.							
	7.							
	8.							
	9.							
	10.							

Fig. 3.

When testing a patient, the tests are made in parallel rows of ten extending from the upper portion of the shoulder to the region of the elbow. In this way five rows of ten each can be put on one arm, each row corresponding exactly to two rows on the trays.

After the skin incision has been made, a drop of pollen extract is dropped on the incision, care being taken not to touch the skin in order to avoid con-

tamination of the pipette. The solution is then rubbed into the incision with a sterile toothpick. This, we believe, offers the simplest and most rapid method possible.

COMMENT

The only disadvantage which occurs to us is the expense of 5 c.c. quantities of 2 per cent extracts of each of the pollens for those who test only occasionally. We believe there is little danger that the solutions will become contaminated from exposure to air on account of the high salt and glycerin content.

The advantages are:

1. Fifty tests can be performed in twenty minutes at most.
2. The technic is extremely simple.
3. No dilution of the extracts occurs as when using the one syringe method of withdrawing solutions from rubber-stoppered vials.
4. Elimination of writing, both on skin and records.
5. While most patients are tested with both trays, it is sometimes desirable to test with trees or grasses and weeds only. By separating them into groups, further time is saved.

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MICROPHOTOGRAPHIC TISSUE REPORTS*

BY ROBERT A. KILDUFFE, M.D., ATLANTIC CITY, N. J.

IT IS possible that the procedure hereafter described may already be in use but as no report of it has been encountered and those who have seen it regard it as, at least, infrequent, it is described herewith for the benefit of those who may be interested.

It consists, in brief, in attaching to the report of histologic tissue examinations embodying the usual gross and microscopic descriptions, microphotographs of characteristic fields showing the salient features upon which the histologic diagnosis was made.

This procedure has the following advantages:

1. It assures the pathologist that the clinician or surgeon will see exactly what the pathologist saw and the exact features of the sections upon which the diagnosis was based.

It is true that the actual slides are available for study by the clinician or surgeon but it is also true that he may not always come to the laboratory at the most convenient time. Moreover, unless he is skilled to some degree in the microscopic examination of tissues, the pathologist may not always be sure that they both are seeing the same field, the same part of the field, or the essential features of the field.

*From the Laboratories of the Atlantic City Hospital.
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The microphotograph, on the other hand, assures all this and permits by means of explanatory legends and corresponding lines upon the print, a clear and unmistakable presentation of the facts.

2. In the event that it is desired to secure additional opinions, the slides sent away may be accompanied by prints of the fields deemed of importance.

3. A tissue report accompanied by microphotographs is a threefold protection in the case of a dissatisfied patient. It protects the pathologist, the surgeon, and the institution by presenting evidence visible to any one and so explaining and justifying the conduct pursued in handling the case.

4. If it is desired to furnish reports to more than one physician concerned in the case, the photographs assure that all will be equally cognizant of the microscopic details of the tissue.

5. If a surgeon or clinician desires to present a case or a series of cases at a staff or other meeting, the photographs are not only useful but time-saving as obviating a long drawn out description.

6. In the course of time a collection of prints is acquired which are useful in the teaching of interns, for example.

7. A collection of lantern slides of rare or interesting sections is readily made for case presentation, teaching, etc.

8. Illustrations for published reports are always available.

It may be objected that the method is expensive, requires skill and experience in photography and a large amount of apparatus, and, hence, is feasible only in large and exceedingly well-equipped laboratories. The purpose of this note, in addition to calling attention to the procedure itself, is to emphasize its relative cheapness and its feasibility for even the small hospital and the small laboratory.

The amount of skill and experience in microphotography which is required is acquired with surprising ease and rapidity.

The question of exposure is, of course, of essential importance, but is not as great an obstacle to the inexperienced as it appears at first glance.

The proper exposure to secure a clear-cut and satisfactory negative is, obviously, influenced by a variety of factors among which may be mentioned the character of the subject to be photographed, the character of the light, the distance from the light and so on.

If, however, the camera, the microscope, and the light source all bear a constant and unchanged relationship to one another, a very little trial suffices to establish a standard exposure producing satisfactory results and from which minor variations can be made as experience dictates as will be described below.

The apparatus used in these laboratories is the Hegener vertical camera manufactured by Zeiss and secured through the A. H. Thomas Company.

The camera is mounted on a heavy base, one side of which accommodates the microscope, the position of which is maintained by a fence and clamps. At the opposite end, a self-contained simplified illuminating apparatus is screwed to the sole-plate.

A tapered column, with a slanting aperture through its plinth for transmission of light to the microscope mirror, is attached to the base between the

microscope and the illuminating apparatus. The vertical motion bar of the camera fits in the bore of the tapered column and is clamped therein by means of a screw. When the latter is released the camera can be swung aside. The eyepiece end of the microscope is then unobstructed, so that the object may be viewed under the microscope, the illumination centered and other adjustments made, which cannot be done satisfactorily while viewing the focusing screen. If desired, the camera can be detached from the upright and used otherwise, for example on a separate foot, without microscope.

A resistance should be used with the lamp in order to provide a means of adjusting the voltage of the lamp terminals exactly to the prescribed value and to reduce the voltage when the full intensity of the lamp is unnecessary, as in the preliminary preparation of the object and in photographing at low magnification. Its use extends the life of the lamp.

The list price of the outfit, without the microscope is $155.50. This outfit takes a 3¼ by 4¼ inch picture which is a satisfactory size for the purpose, and if the fields photographed are well selected, it shows all that is necessary.

The method of procedure is as follows: A satisfactory field is found as usual and the microscope then clamped in position on the camera base, or the microscope may be first placed in position, the camera swung aside, the appropriate field found, and the camera then swung into place.

With the shutter open and the ground glass screen in place, the light is adjusted by means of the rheostat, the diaphragm on the light column and the microscope mirror until the field is evenly and brightly illuminated. The field is then focused. The ground glass screen is then replaced by one of plain glass, the light cut down with the rheostat, and a finer focus obtained by means of a focusing glass. The shutter is then closed.

A few trials are necessary to secure the "standard exposure." If one has had no preliminary experience the simplest method is as follows:

1. Do not, in the case of tissues, attempt to take a picture with the full intensity of the light.

2. All exposures are time exposures.

3. The brighter the light, the shorter the exposure and vice versa.

4. Close the shutter, remove the glass screen, and insert the plate or film holder.

5. Secure the maximum diminution of light by means of the rheostat and then move the rheostat slide along until about one-third the maximum light intensity is obtained, or, about one-third the length of the rheostat.

6. Withdraw the slide and expose the plate or film for an arbitrary number of seconds. Then close the camera shutter, push in the slide so as to leave only one-fourth the film or plate exposed and again expose for the same time. This procedure is repeated until all four segments of the film or plate have been exposed.

The four segments of the film or plate have thus been exposed for the following times: The whole film or plate received at first two seconds, for example, one-fourth is covered and receives no longer exposure. The three-fourths then receives a second exposure of two seconds, or four seconds in all. A second fourth of the film or plate is then covered with the slide, and

the remaining half again exposed and so on. The exposures of the four segments then are two, four, six, and eight seconds.

When the plate or film is developed, if none of the strips are satisfactory, the procedure is repeated starting with greater or less exposure times as indicated. Once an exposure giving a clear-cut negative is obtained, this point on the rheostat is noted and used, thereafter, for tissue photographs as the "standard exposure time."

The same procedure is followed for oil immersion and high power work as, obviously, the amount of light passing through decreases in proportion to the lens and more illumination or increased exposure or both may be required.

While this crude method will give surprisingly good results in the hands of the inexperienced, as experience accumulates minor variations will be indicated in accordance with the character and density of the sections and so on.

As all the sections are stained a color filter is necessary to produce the best results as to contrast.

A very excellent, satisfactory and simple filter may be prepared as follows:

Copper sulphate	175 gm.
Potassium chromate	17 gm.
Sulphuric acid	2 c.c.
Water to make	500 c.c.

This solution is placed in the glass cell of the camera. An equally satisfactory color filter for general use is the Wratten K3.

Either plates or film may be used, the Wratten M plate and Eastman panchromatic film being the best.

Developing and printing may be equally simplified and an elaborate darkroom equipment is unnecessary.

In these laboratories because of lack of space a small closet is utilized as a dark room.

It was made light-proof with a red light and, as required by the panchromatic film and plate, a No. 3 Wratten safe-light, a fan for ventilation through several small "windows," and a printing machine. Developing and fixing are done in trays. Formulas for these solutions accompany the films and plates or the prepared powders made for x-ray developing may be used.

These come in sealed cans in quantity sufficient to make 1 to 3 gallons of solution and require only to be dissolved in the appropriate amount of water. They are suitable for both negatives and prints.

Once the apparatus is purchased, which may be done for $200, the expense is negligible. A little more work and a little delay in furnishing the written and illustrated report is entailed but more than compensated for by the results. It is unnecessary to photograph all tissues, only those in which malignancy is found or suspected and not found or those which possess particular interest.

ATLANTIC CITY HOSPITAL.

A SIMPLE DIFFERENTIAL DIAGNOSTIC PROCEDURE IN DISEASES OF THE HEMATOPOIETIC SYSTEM*

By Arthur Weiss, B.S., M.D., New York City

A SIMPLE, BUT VALUABLE DIFFERENTIAL DIAGNOSTIC HEMATOLOGIC PROCEDURE

FOR the past four years, I have been employing both in the hospital and in my practice a simple device that I have found to be of definite value in the differential diagnosis of hematologic conditions. It consists of a piece of capillary tubing about 3 to 4 inches long that is formed into a U with the bunsen flame.

Technic: The tip of a finger or the lobe of an ear having been cleansed and dried, is pricked so that it gives an easy steady flow to light pressure. The U-tube is held horizontally, one of the arms touching the blood drop. As the blood enters the capillary tube, the position of the tube is gradually

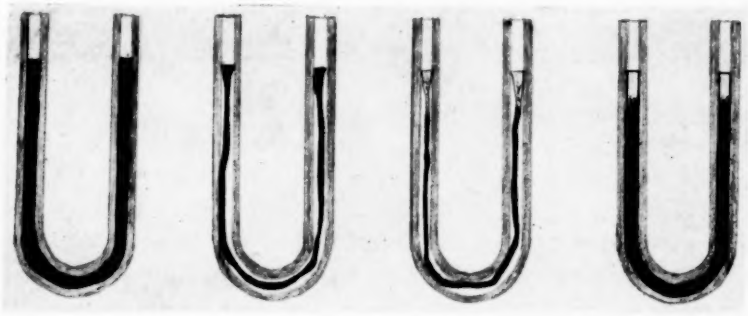

Fig. 1. Fig. 2. Fig. 3. Fig. 4.

Fig. 1.—Normal five minutes after filling.
Fig. 2.—Normal retraction three-fourths to one hour after.
Fig. 3.—Retraction in case of severe anemia or infection.
Fig. 4.—No retraction one to twenty-four hours after filling, as in essential purpura.

changed toward the vertical to facilitate the further advance of the blood column until the tube is about three-fourths full. One must be careful that no air bubbles enter to interrupt and break the continuity of the blood column. It is very important that the finger prick be sufficiently deep, for if the flow is poor the blood will clot before the U-tube is filled. When the tube is full, it is placed in a vertical position.

Findings: Normally, it will be found that between three and five minutes the blood will clot and will not move when the position of the tube is altered (Fig. 1). In about one-half hour the margins of the clot will begin to retract from the sides of the tube, and between one hour and one and a half hours the retraction will be complete as in Fig. 2, showing an absolutely clear transparent and colorless serum. From these findings we will then find that the

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coagulation time, the bleeding time, clot retraction, platelet count, Van den Bergh test and Icteric Index will be normal.

Under pathologic conditions, however, we will find the following:

1. In *severe infections*, there will be due to an increased globulin content of the blood plasma, a sedimentation of the erythrocytes depending on the severity of the infection (Fig. 3). Also due to the increased blood platelets found in severe infections, it will be found that the clot retraction will begin earlier, in fifteen minutes.

2. In *hemophilia*, it will be found that instead of the clot being firm in three to five minutes, any altering of the position of the tube will cause the blood column to move. Movement of the blood column can thus be caused after ten to sixty minutes have elapsed (Fig. 5).

3. In *suspicious secondary anemias*, depending on their severity, there will be a sedimentation of the erythrocytes as in Fig. 3, and early retraction of the blood clot if the anemia is posthemorrhagic. Serum will be clear transparent

Fig. 5.—No coagulation five to thirty minutes after filling. Hemophilia.

and colorless. If the anemia is marked there will be a perceptible increase in the amount of serum as compared with the blood clot, indicating a decreased volume index.

4. In the hemolytic anemias, *primary pernicious anemia*, and *hemolytic icterus*, there will be primarily, depending on the severity of the anemia, sedimentation of the red blood cells, secondarily and most outstanding, will be a definitely yellow to golden serum depending on the amount of bilirubin and hematin present in the blood serum. The U-tube in a case of obstructive jaundice would also show the same serum color; but in the latter case the history, physical findings, and other laboratory data would definitely place it.

5. In *purpura hemorrhagica* (thrombopenic and thrombolytic) the findings are more or less diagnostic. If there was no anemia present, the U-tube at the end of twenty-four hours would look like Fig. 1, if anemia were present, it would look like Fig. 4. There would be absolutely no retraction of the clot. After splenectomy the U-tube will show retraction of the clot returning very quickly. Polycythemia although without retraction can be differentiated by count.

Conclusion: Thus by this simple procedure, necessitating nothing but a piece of capillary tubing and blood gotten by prick of a finger, you can before

having examined cell morphology glean very many useful facts that can act as a guide as to what other tests you should undertake. After examining the cell morphology you can with the aid of the U-tube definitely differentiate between a primary or secondary anemia, or a symptomatic or thrombolytic purpura. Where many preoperative cases await hematologic workup, it will be found to be a very useful aid. This procedure does not attempt to supersede the actual taking of the bleeding time, coagulation time, platelet count, and van den Bergh, but it acts as a guide, and also corroborates them.

A STUDY OF THE MYERS-WARDELL METHOD FOR THE DETERMINATION OF CHOLESTEROL*

By Frances Krasnow, Ph.D., and A. S. Rosen, Phar.D., New York

INQUIRY into the detailed changes of the blood lipins during syphilitic infection necessitated the use of an accurate technic for the determination of cholesterol.† We chose that recommended by Myers and Wardell as the most desirable.‡

However, certain difficulties were encountered. For example, it was found inconvenient to employ a crucible or a beaker for mixing the blood and plaster of Paris. Then, the different market plasters varied so that a suitable grade had to be located or a method for standardizing the available ones evolved. Tests during the last four years show that Clover Leaf Plaster: X Standard Set, obtained from H. B. Wiggin's Sons Company, Bloomfield, New Jersey, is excellent for the purpose. Moreover, the suitability of a brand may be ascertained by comparing it in parallel determinations with $CaSO_4.2H_2O$ (Merck Blue Label) dried to constant weight at 160° C.

Another source of great concern was the period and temperature for drying the blood-plaster mixture, because cholesterol decomposes at high temperatures or even by prolonged heating at low temperatures. Experiments indicate that the safe interval is two hours. If the period is shortened the color develops with a brown tint although there is no loss in cholesterol. Longer periods make for poor color development and are also responsible for low values, the loss varying directly with the amount of heating. Thus, for two, four, six, eight, and twenty hours we obtained respectively 186, 167, 151, 137, and 135 mg. of cholesterol per 100 c.c. of whole blood.

Finally, filter paper thimbles were found more convenient and less expensive than extraction shells. For this purpose an ordinary good grade filter paper (5 inches in diameter) is extracted for six hours with $CHCl_3$ in a Soxlet apparatus and dried thoroughly. It is then folded as shown in Chart I.

The filter paper is folded into form DCE as in figure (a) of Chart I, and a

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*From the Laboratory of Biological Chemistry of Columbia University at the College of Physicians and Surgeons.

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†The results of this study will appear in the Archives of Dermatology and Syphilology in a series of articles. They are based on the findings for 99 normal individuals and 200 patients suffering with syphilis.

‡Myers, V. C., and Wardell, E. L.: J. Biol. 36: 147, 1918.

crease made along AB so that DB is slightly less than BE and CA about 2/3 AE, giving flap BEA in figure (b). Then DCAB consists of four layers, DCAB. With the flap facing toward you the farthest layer DCAB is separated from the other three layers, thereby forming a thimble which fits snugly into a glass thimble (2¼ inches high by 1 inch diameter). Instead of suspending the thimble from the condenser, it is set into a Soxlet apparatus. The latter was so made that the ground glass end of its extraction chamber fits inside of the condenser and the siphon tube is not more than 1¼ inches high.

Briefly then, the details of the procedure as adopted are:

The blood (5 c.c.) is taken into a tube containing the dried residue of 2 c.c. of K oxalate (1 per cent). If not used immediately, the blood should be kept in the ice chest. The determination ought not to be delayed more than twenty-four hours.

Whole blood (1 c.c.) is pipetted with an Ostwald-Folin pipette into a casserol of about 75 c.c. capacity and covered with 7 gm. of plaster of Paris. The mixture is triturated well with a small glass pestle until a uniformly pink powder is obtained. All substance is thoroughly loosened from the sides and

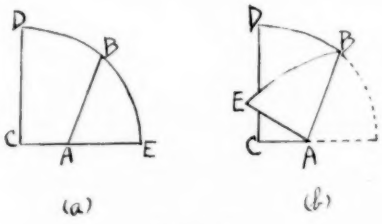


Chart I.

bottom with a flexible steel spatula. The dish is placed in the drying oven at 95° to 100° C. for two hours. After cooled, the plaster mixture is transferred quantitatively with the aid of black glazed paper to a fat-free filter paper thimble contained in a glass thimble having several holes in its bottom.

The whole is then placed into the extraction chamber of a Soxlet apparatus of the type described above and set into the extract flask which contains 35 c.c. of $CHCl_3$.* Extraction is allowed to proceed for forty-five minutes. The flask containing the extract is removed from the hot plate when the level of the $CHCl_3$ in the extraction chamber is one-half the height of the siphon tube. The extract is transferred to a 25 c.c. volumetric flask. To this are added the washings carried out with the $CHCl_3$ which remained in the extraction chamber. After the $CHCl_3$ containing the extracted cholesterol is cooled to room temperature or to the temperature at which the apparatus has been standardized, the volume is made up to mark.†

One-tenth c.c. of H_2SO_4 (sp. gr. 1.84), 2 c.c. of acetic anhydride (Merck Blue Label)‡ and 5 c.c. of the extract are pipetted into an ordinary test tube

*The $CHCl_3$ is dried with anhydrous Na_2SO_4 or plaster of Paris by shaking several times daily for two weeks and distilled slowly so that the condensed $CHCl_3$ enters the receiver drop by drop.

†The $CHCl_3$ extract keeps apparently unaltered for a long period when protected from sunlight.

‡The H_2SO_4 and acetic anhydride should not be mixed long before the addition of the extract.

(6 inches by ¾ inch), shaken and placed in the dark for ten minutes. The color developed is matched against a naphthol green B standard. A 0.1 per cent solution of this in distilled water serves as the stock concentration. When needed, 1 c.c. of the latter is diluted with 19 c.c. of water.* If the proper quality of the naphthol green is obtained, this solution set at 15.5 mm. on the colorimeter scale will just match 0.0004 gm. of pure cholesterol in 5 c.c. of $CHCl_3$ at 15.

GLYCERIN PRESERVATION OF SEROLOGIC TEST MATERIALS, AND THE INFLUENCE OF HEAT ON GLYCERINATED WASSERMANN-POSITIVE SERA†

By Frances Perstein and Hamilton R. Fishback, Chicago, Ill.

THE preservation of human sera for future serologic tests and controls is of importance in the laboratory in which the quantity of serum available may be rather small. The question may be still more urgent if the serum is being used for other than routine work.

Of the methods in use for keeping of sera, glycerin preservation[1] seems well adapted for such purpose. Wassermann amboceptor is quite commonly preserved with glycerin.[2] Besides preserving sera for Wassermann and other complement-fixation tests, it may be used in sera for tests involving agglutinins, precipitins, or lysins.[3] We have used it regularly in keeping spinal fluids for the Lange test. The specificity and delicacy of the reaction are not lessened. Also glycerin may be added to colloidal solutions commercially to render them somewhat more stable, and without injuring their reactive qualities.

The glycerin should be of standard chemical purity. It is a stable alcohol, and is not altered by long standing. Ordinary glycerin will be found to contain traces of the alkali used in saponification, or fatty acids, acrolein or other organic materials. These impurities may be strongly anticomplementary and may tend toward deterioration of the syphilitic reagin in the preserved serum. Sterilization of the glycerin is perhaps desirable theoretically, but whatever bacteria may be carried over in unsterilized glycerin do not grow in the 50 per cent concentration used in preserving sera.[4]

Chart I

Effect of Glycerin on the Sensitiveness of the Wassermann Test

Case No.	Serum	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1 c.c.
49928-D	Non-Glycer.	0	3	4	4	4	4	4	4	4	4
	Glycer.	0	0	4	4	4	4	4	4	4	4
52034-D	Non-Glycer.	2	3	4	4	4	4	4	4	4	4
	Glycer.	0	4	4	4	4	4	4	4	4	4
51635-D	Non-Glycer.	0	0	2	4	4	4	4	4	4	4
	Glycer.	0	0	0	4	4	4	4	4	4	4
51847-D	Non-Glycer.	0	4	4	4	4	4	4	4	4	4
	Glycer.	3	4	4	4	4	4	4	4	4	4

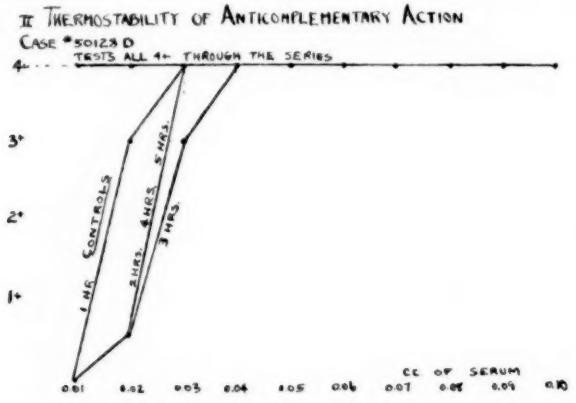
*Diluted naphthol green B deteriorates on standing. All market grades of naphthol do not match the color developed by cholesterol. In emergency, it may be necessary to match against cholesterol.

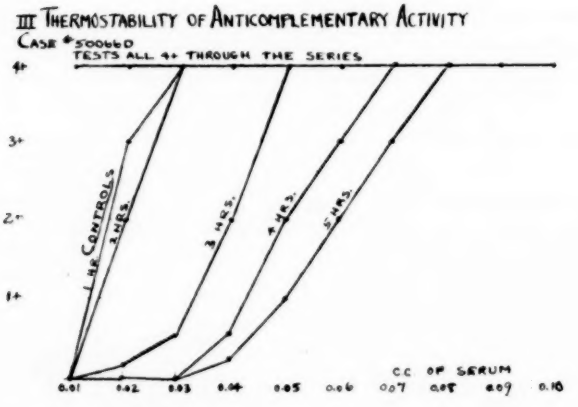
†From the Pathology Laboratory of Wesley Memorial Hospital, Chicago, and the Department of Pathology, Northwestern University Medical School.

Received for publication, October 5, 1928.

The effect of glycerin upon the Wassermann reacting body is a strengthening one. That it is more than nonspecific absorption of complement is indicated by the persistence of the stronger Wassermann reaction after removal of the anticomplementary activity by titration.

In Chart I, the glycerinated serum is 50 per cent glycerin. The reactions given after the addition of glycerin are seen to be constantly somewhat stronger. One Wassermann modification for which high sensitiveness is claimed[5] has for one of its factors the addition of glycerin. A favorable influence upon a flocculation test for syphilis[6] is also reported.



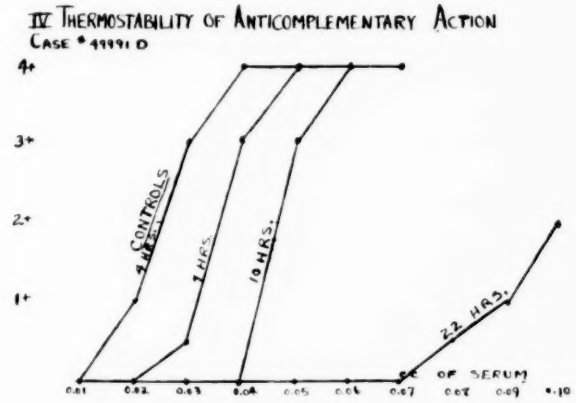


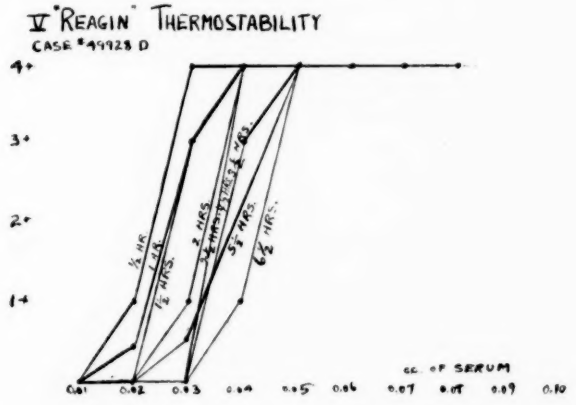
An objection to the use of glycerin as a preservative for Wassermann sera is that it may render them strongly anticomplementary. This effect may be removed from many sera by heating,[1] but returns again on standing. In many others, thirty minutes heating at 56° C. weakens but does not remove the anticomplementary effect. It may be also quite heat resistant.

A series of tests was run with prolonged heating of Wassermann positive sera inactivated for thirty minutes at 56° C. and preserved by the addition of glycerin to 50 per cent strength, to determine the effect of heat upon those sera with somewhat more thermostable anticomplementary activity. In these tests the serum sample was divided into a number of portions, according to

the amount of serum available. Each portion was put into a tightly closed tube, and they were all heated in a closed water-bath at 56° C. At the end of each time interval a portion was removed and used at once in the Wassermann titration test.

The results from each separate portion are plotted as to amount of serum and strength of reaction in each tube of the titration series, and the portions are marked according to the length of time of heating. In Charts II and III curves are given for both test and control tubes, while in Chart IV only con-





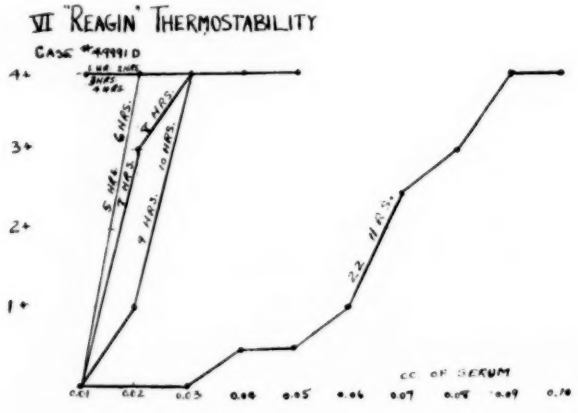
trol tube results are plotted. It is noted that the curves for the tests in Charts II and III are identical, since they are 4+ for all ten tubes in each portion. The curves for the control tubes are sometimes superimposed, as in the two-hour, four-hour, and five-hour heated portions of Chart II, and it is evident that they all have a common level for the remainder of the curve after the 4+ value is reached.

In Charts II, III, and IV, long heating is found to depress the nonspecific complement binding action of these sera very slowly, so that complete binding of complement is found with less than 0.05 c.c. of serum in sera heated varying lengths of time up to ten hours. No evidence is found of destruction of serum components by heat with possible increase of anticomplementary power.

We have found that the method of elimination of anticomplementary activity by titration is effective here also.[7] When the amount is established which is still Wassermann positive and free from anticomplementary action, then this amount may be used in future tests. If the titration is carried low enough there is usually a range of three or more positive tubes without anticomplementary reaction in the controls.

The resistance of the Wassermann reagin to heat was also determined in a series of tests with glycerin preserved sera. The procedure was similar to that described above.

In Chart V, using a serum of lower Wassermann potency, it will be noted that the values after one hour, and six and one-half hours heating, lie only 0.02 c.c. apart, and that a 4+ reaction is given after the final heating by 0.05 c.c. Since the serum samples are 50 per cent glycerin the amount of serum is actually only 0.025 c.c. In Chart VI, a 4+ reaction is obtained by 0.005 c.c.



serum after four hours of heating, and by 0.045 c.c. after twenty-two hours heating. The decrease in Wassermann titer is gradual, but slight in amount. In Charts II and III also it is seen that all tests remain 4+ through the series, with a total of five hours heating in each serum. The smallest amount used in each titration is 0.005 c.c. of serum.

The amount of heat resistance of the Wassermann reacting body of these glycerin preserved sera is striking. Other work has been published using a thirty-minute period of heating of glycerinated and nonglycerinated serum, with results slightly in favor of the glycerinated serum, although usually neither gave as strong reactions as the unheated serum.[8] Many workers are agreed that thirty minutes of heating may weaken the Wassermann reaction significantly,[9] although no one seems to have measured such change by the quantitative titration method. Not all sera react alike to heat, and the reasons for such variation are as yet only speculative. If the Wassermann reaction is effected because of a particular physicochemical state of lipoids or lipoid-protein combination in the blood, then glycerin may stabilize such a reactive state as to the influence of both time and heat.

CONCLUSIONS

Glycerin is a satisfactory preservative for the reacting materials of many serologic tests.

The anticomplementary function acquired by many Wassermann positive sera on the addition of glycerin may be quite heat resistant. In such sera the anticomplementary action may be eliminated by titration.

The Wassermann reacting body in glycerinated positive sera was found to survive prolonged heating at 56° C.

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A SIMPLIFIED PORTABLE APPARATUS FOR THE DETERMINATION OF THE CARBON DIOXIDE CAPACITY OF PLASMA*

By William Z. Fradkin, A.B., M.D., Brooklyn

THE cost and size of the Van Slyke CO_2 apparatus has made it difficult for the general practitioner to use it as a practical clinical aid in the detection of acidosis or alkalosis. Its usefulness has been restricted mainly to hospital laboratories. With this fact in mind I have devised a simpler apparatus† which is small, portable, comparatively cheap, and easy to handle. It is not intended to replace the present Van Slyke apparatus, but rather to fill a need in the general practitioner's laboratory.

THE APPARATUS

The apparatus as shown in Fig. 1 consists of a 25 c.c. chamber with two one-way stopcocks at the top and side, a 0.5 c.c. scale on the upper stem divided into 0.01 c.c. divisions, and a side arm for the reception of plasma and reagents. The bottom of the apparatus is connected by a pressure rubber tube to a leveling cup filled with mercury. The chamber is held in place above and below by screw clamps, the jaws of which are lined with pads of rubber. Capillary (x) is used for the removal of fluids from the apparatus. The entire apparatus is mounted on a heavy metal tube which slides up and down a metal rod fastened to the bottom of the cabinet. The thumb screw in front

*From the Department of Pathology of the Jewish Hospital of Brooklyn.
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†The apparatus is manufactured by the Emil Greiner Company, 55 Vandam Street, New York City.

is used to clamp the apparatus at any desired level. In addition to the chamber and leveling cup the cabinet also contains reagent bottles, a pipette, a medicine dropper, and a tube with lubricant.

REAGENTS

1—12 per cent sulphuric acid
2—Caprylic ethyl alcohol
3—Distilled water
4—Redistilled mercury

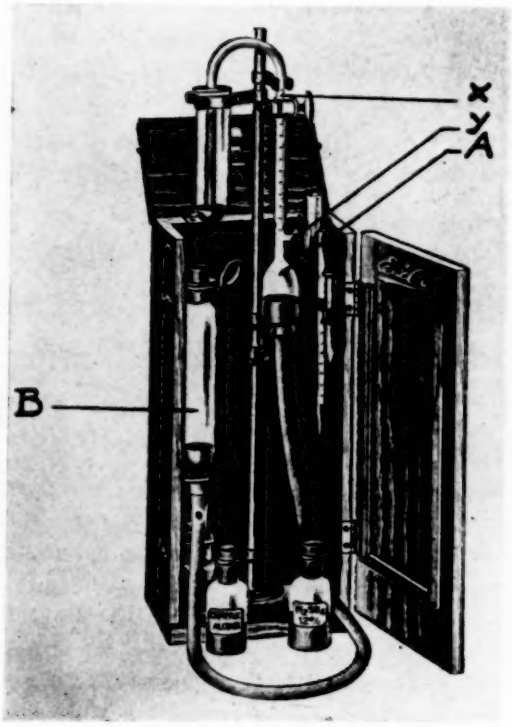

Fig. 1.

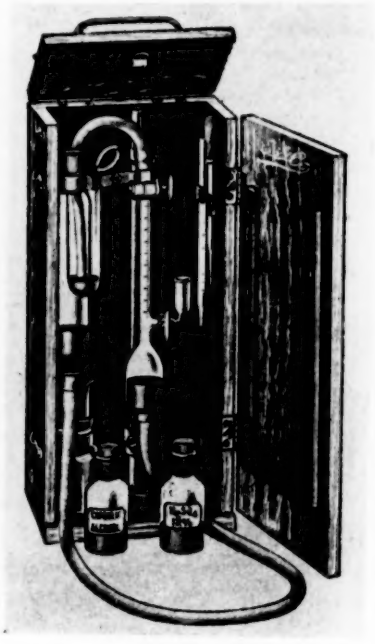

Fig. 2.

THE DETERMINATION

Raise the apparatus to position (A) by loosening the thumbscrew. Produce a vacuum by lowering the mercury leveling cup. Raise the leveling cup. If the apparatus is tight and gas free the mercury will strike the upper cock with a sharp click. If this click is not heard allow the accumulated gas or fluid to escape by opening the upper cock and repeat lowering and raising the mercury leveling cup until the click is heard. Introduce 0.5 c.c. of distilled water into the side arm with a pipette. Transfer 0.5 c.c. of blood plasma into the side arm taking care that the tip of the pipette dips below the surface of the water. With the mercury leveling cup in position (B) rotate stopcock slowly until the fluid in the side arm begins to enter the chamber and displaces the mercury from above. No harm is done if the mercury column in

the chamber is broken by the entrance of the solution. Run in a very small drop of caprylic alcohol into capillary (y). Finally 0.2 c.c. of 12 per cent sulphuric acid is run in. Then seal the stopcock by running a drop of mercury through the capillary (y). Twelve per cent sulphuric acid is used in order to keep the quantity of fluid to a minimum. Lower the mercury leveling cup until the mercury in the chamber reaches the mark 25. It may be necessary to tap the upper stem with the finger so that all the mercury drops to the bottom. Tip the box gently back and forth for two minutes thus agitating the solution in the chamber. The time of shaking may be controlled by counting slowly 120 numbers which approximates two minutes. The leveling cup with the left hand is then raised rather rapidly so that it reaches the level of the mercury meniscus in the stem. The volume of gas is read at once in order to avoid reabsorption of the carbon dioxide.

The results are calculated for 100 c.c. of plasma by using Table IV which is the same as that given by Van Slyke, except that it has been modified to correspond to half the quantity of plasma used.

DRAWING BLOOD SAMPLES

Place in an ordinary tube 4 drops of 25 per cent potassium oxalate solution. Puncture a vein with an ordinary Wassermann needle and allow the blood to flow into the tube until about 5 c.c. is obtained. Cork the tube immediately. Invert the tube two or three times and let the plasma separate by gravity. Then draw off the plasma and analyze. It is not necessary to saturate the plasma with air containing CO_2 if the analysis is done without any delay.

TABLE I

COMPARISON OF CO_2 DETERMINATION WITH THE SIMPLIFIED APPARATUS AND THE VAN SLYKE APPARATUS

	SIMPLIFIED	VAN SLYKE	VOLUME PER CENT DIFFERENCE
Blood samples from patients in acidosis:			
1	28.3	30.3	2.0
2	22.6	22.6	0.0
3	18.8	16.8	2.0
4	39.1	38.1	1.0
5	36.1	37.1	1.0
Blood samples from patients without symptoms of acidosis.			
1	58.8	57.4	1.4
2	54.5	55.5	1.0
3	64.2	63.2	1.0
4	57.4	55.5	1.9
5	52.6	52.6	0.0

DISCUSSION OF TABLE I

Table I shows only slight differences between the two apparatus. Considering the original Van Slyke as the standard, the simplified apparatus is accurate within 1.5 volumes per cent.

CLEANING

The chamber is cleaned with two washings of distilled water, about 5 c.c. each, admitted through the side arm and expelled through the stem by opening the upper stopcock and raising the leveling cup.

LUBRICATION

Both stopcocks must be lubricated occasionally in order that they may turn smoothly and also to avoid any possibility of a leak.

TABLE II

REPRODUCIBILITY OF THE SIMPLIFIED AND VAN SLYKE APPARATUS

	SIMPLIFIED	VAN SLYKE
On one blood sample from a patient in acidosis:		
1	16.8	17.8
2	16.8	16.8
3	16.8	17.8
4	16.8	15.1
5	15.1	15.1
	Average 16.4	Average 16.5
On one blood sample from a patient without acidosis:		
1	57.4	55.5
2	56.7	54.5
3	56.7	53.6
4	55.5	54.5
5	54.5	52.6
	Average 56.1	Average 54.1

DISCUSSION OF TABLE II

Table II shows more consistent results in reproducibility with the simplified apparatus than with the Van Slyke.

TABLE III

ANALYSIS OF STANDARD CARBONATE SOLUTION BY THE SIMPLIFIED APPARATUS
(Solution Contained 52.9 Volumes of CO_2 per 100 c.c. of Solution by Calculation)

1	55.5
2	53.6
3	55.5
4	53.6
5	55.5
6	53.6
7	53.6
8	53.6
9	54.5
Average	54.3
Standard	52.9
Volume difference	1.4

DISCUSSION OF TABLE III

Table III shows a number of analyses on a standard carbonate solution. The results are within the limit of error of a determination. There is a 2.6 per cent error and only a difference of 1.4 volumes per cent.

TABLE IV

TABLE FOR CALCULATION OF CARBON DIOXIDE CAPACITY OF PLASMA
(In c.c. of CO_2 Chemically Bound by 100 c.c. of Plasma)

0.100	9.4	0.300	47.5
0.105	10.1	0.305	48.7
0.110	11.0	0.310	49.7
0.115	12.0	0.315	50.7
0.120	13.0	0.320	51.6
0.125	13.9	0.325	52.6
0.130	14.9	0.330	53.6
0.135	15.9	0.335	54.5
0.140	16.8	0.340	55.5
0.145	17.8	0.345	56.7
0.150	18.8	0.350	57.4
0.155	19.7	0.355	58.4
0.160	20.7	0.360	59.4
0.165	21.7	0.365	60.3
0.170	22.6	0.370	61.3
0.175	23.6	0.375	62.3
0.180	24.6	0.380	63.2
0.185	25.5	0.385	64.2
0.190	26.5	0.390	65.2
0.195	27.5	0.395	66.1
0.200	28.4	0.400	67.1
0.205	29.4	0.405	68.1
0.210	30.3	0.410	69.0
0.215	31.3	0.415	70.0
0.220	32.3	0.420	71.0
0.225	33.2	0.425	71.9
0.230	34.2	0.430	72.9
0.235	35.2	0.435	73.9
0.240	36.1	0.440	74.8
0.245	37.1	0.445	75.8
0.250	38.1	0.450	76.8
0.255	39.1	0.455	77.8
0.260	40.0	0.460	78.7
0.265	41.0	0.465	79.1
0.270	42.0	0.470	80.5
0.275	42.9	0.475	81.6
0.280	43.9	0.480	82.6
0.285	44.9	0.485	83.6
0.290	45.8	0.490	84.5
0.295	46.8	0.495	85.5
		0.500	86.5

SUMMARY

A simplified portable, cheap and practical apparatus is described for the rapid determination of carbon dioxide capacity of blood plasma. The analysis may be completed at the bedside within a few minutes after the separation of the blood plasma. The results are accurate to within 2.6 per cent of the amount determined.

Further research is being done with this apparatus to make it suitable for other blood chemical tests by the gasometric method.

I wish to express my thanks to Dr. Max Lederer, Attending Pathologist, for his valuable suggestions and encouragement; also to Mr. I. F. Gittleman for checking up the accuracy of the apparatus.

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2. Van Slyke, D. D.: J. Biol. Chem. **30**: 347, 1917.
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HINTS TO LABORATORY TECHNICIANS*

By Aaron E. Parsonnet, M.D., and Jeannette Dupuis, Newark, N. J.

OF ESPECIAL interest to those who have small laboratories in which every available space must be utilized to good advantage will be this suggestion for holding 50 c.c. and 100 c.c. Erlenmeyer flasks.

Stout wire hooks such as are used in wardrobes can be screwed into a wall (see Fig. 1) each making an ideal rack for two flasks if they are set bottom side up. Hooks were placed about six inches apart, one above the other, making a neat and convenient array of flasks.

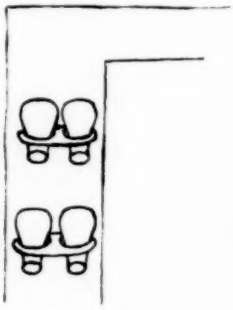

Fig. 1.

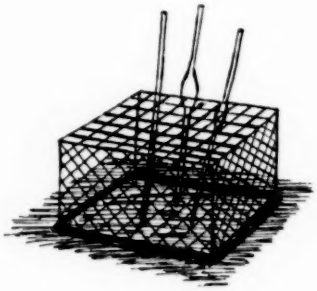

Fig. 2.

Volumeteric pipettes such as those one uses in blood analyses are less apt to break and are more easily kept in order if they are held between the mesh of an inverted test tube basket (see Fig. 2). A layer of cotton and gauze may be made for the top of the basket, which, when inverted, serves as an ideal pad on which the wet pipettes will drain quickly. In this way one may easily classify pipettes of various capacities.

In the Folin-Wu method of blood analyses a good suggestion is to have made 14 c.c. and 35 c.c. volumeteric pipettes which may be used for measuring the seven volumes of water in preparing the blood filtrates. In this manner one is certain of accurate measurement and avoids any possible chance of error.

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STUDIES ON ANTIGEN FOR THE KAHN TEST*

III. The Correction of Antigen

By R. L. Kahn, Grace Lubin, and Elizabeth McDermott, Lansing, Mich.

IN A PREVIOUS article from this laboratory[1] it was shown that the standardization of antigen for the Kahn test comprises three steps. The first step is the titration of the antigen, or the determination of the proportion of antigen and salt solution to be employed in preparing the antigen suspension for the serum tests. The second step comprises a determination of the sensitiveness of the antigen as compared with the sensitiveness of standard antigen, comparative tests being carried out with the two antigens, using syphilitic and nonsyphilitic sera. The final step is the correction of oversensitive or undersensitive antigens to the standard degree of sensitiveness. The aim of this article is to correlate the theoretical and practical aspects of antigen correction, and to present in detail the methods for correcting antigens to standard requirements.

THEORETICAL ASPECTS OF ANTIGEN CORRECTION

In order to present as clearly as possible the principles of antigen correction, it will be well to summarize briefly some of the facts that we have accumulated regarding antigen. Since the titration process is the first step in the standardization of antigen, we shall first touch upon this phase of the problem, stressing especially the relation between antigen sensitiveness and the relative amount of salt solution used in preparing the antigen suspension.

It will be recalled[2] that the titer of an antigen represents the smallest amount of salt solution which, when mixed with 1 c.c. of antigen, results in a suspension that can be dispersed by additional salt solution or by negative serum, forming a clear opalescent solution. During our early studies on the Kahn test, we believed that the inherent differences in the lipid concentration of different antigens would automatically become neutralized in the antigen-salt solution suspensions by corresponding variations in the titer, and that titration would thus be the only necessary procedure in the standardization of antigen. This view was prompted by the observation that low lipid concentration is usually associated with a low titer, and that vice versa, highly concentrated antigens have a relatively high titer. It was assumed that uniformity in the concentration of lipids in the suspension would induce uniformity in sensitiveness in the serum reaction, and it was further assumed in the early studies that dilution with salt solution would, in general, affect the antigen somewhat similarly to dilution with alcohol. Thus, for example, it was supposed that if Antigen A had a titer of 1 + 1.4 and Antigen B a titer of 1 + 0.7, Antigen A, which was richer in lipids than Antigen B would, when mixed

*From the Bureau of Laboratories, Michigan Department of Health, Lansing. July, 1928. Received for publication, July 17, 1928.

with salt solution at its relatively high titer, be closely parallel in sensitiveness to Antigen B at its relatively low titer. Experience with a large number of antigens indicates, however, that the titer cannot be depended upon as an automatic corrective of antigen sensitiveness. In the supposed case of Antigens A and B, for example, strict adherence to their respective titers may not neutralize basic differences in sensitiveness. It is this fact that necessitated the evolvement of several methods of antigen correction.

It is of interest to note that the titer of an antigen does not invariably increase with an increase in lipid concentration. Indeed, if the concentration of an antigen is increased by means of an additional amount of its own lipids (see page 988), the amount of salt solution required for the titer tends in some cases to be reduced. For example, if an antigen originally has a titer of 1 + 1.4, the titer might be 1 + 1.2 after the antigen has been concentrated by 10 or 20 per cent.

Let us consider the effects of varying the amounts of salt solution used in preparing the antigen suspension. If the amount of salt solution used is 0.1 or 0.2 c.c. less than that indicated by the titer, the particles of the suspension will not be completely dispersed by the addition of a relative excess of salt solution or nonsyphilitic serum, and the mixture of suspension and salt solution or of suspension and negative serum will contain visible particles or will appear turbid due to the presence of particles that are just below the visible range. Inasmuch as the conditions of the test demand that the negative reactions appear clear and opalescent, the use of less salt solution than that indicated by the titer is thus precluded. If, on the other hand, a series of antigen suspensions is prepared with amounts of salt solution progressively greater than that indicated by the titer, these suspensions, which give progressively clearer opalescent solutions when mixed with salt solution or with negative serum, will also be progressively less and less sensitive when tested with syphilitic serum. In other words, an antigen suspension is not fit for use in the Kahn test when prepared with less salt solution than that indicated by the titer, and it is more sensitive when prepared according to the titer than when prepared with an amount of salt solution greater than that required by the titer. This latter relation provides a method for the correction of antigens that are more sensitive than standard. Thus, if the titer of the more sensitive antigen is, let us say, 1 + 1.2, a suspension prepared by mixing 1 c.c. antigen with 1.5 c.c. salt solution might be sufficiently reduced in sensitiveness to be comparable with standard antigen. This method of correcting oversensitive antigens appears to be less readily controlled than certain other methods to be discussed presently, and the method has not been adopted in this laboratory excepting for antigens that are but very slightly oversensitive, which may be brought to standard sensitiveness by using only 0.1 or 0.2 c.c. more salt solution than that indicated by the titer.

It has been shown elsewhere[3] that the sensitiveness of an antigen is closely related to its lipid concentration. An antigen shows maximum sensitiveness only at a certain concentration, referred to as optimum for that particular antigen. If, when an antigen is at its optimum concentration, the concentration is either increased (by the addition of lipids from the same antigen) or decreased (by dilution of the cholesterolized antigen with cholesterolized

alcohol), the sensitiveness of the antigen is correspondingly decreased. When the increase or decrease in concentration is excessive, antigenic sensitiveness may be reduced to zero.

Our experiments indicate that this relation between lipid concentration and sensitiveness applies to all antigens prepared according to standard requirements, irrespective of whether the source of the lipids is beef, sheep, or pig heart. It has been found, however, that the actual value of the maximum sensitiveness and of the optimum concentration varies for different antigens. At one time we believed that undersensitiveness in antigens was invariably due to deficient concentration of lipids and could always be corrected by increasing the concentration of the antigen with its own lipids. When this method of correcting undersensitive antigens is attempted, however, the sensitiveness of the antigen is found to be almost always still further decreased. This would indicate that some other factor besides total lipid concentration controls antigen sensitiveness, and suggests that we are probably dealing with at least two types of lipid substances in antigens, an antigenic substance and a substance inhibitory to precipitation. In other words quality as well as quantity of heart muscle extractives determines antigen sensitiveness. Thus, in the correction of antigens we must keep in mind the two variables, ratio of antigenic to inhibitory lipids initially extracted by the alcohol in the preparation of a given antigen, and total concentration of heart muscle extractives. Should the original extract contain a favorable ratio, the antigen will be likely to have a relatively high sensitiveness unless its total concentration is too high or too low. If, on the other hand, the ratio of antigenic to inhibitory substances extracted in a given case is unfavorable, the sensitiveness of the antigen is apt to be low originally, and to become still lower if to the antigen is added an additional amount of its own lipids. It is of interest to note that the sensitiveness of an inherently undersensitive antigen may be considerably increased by adding to the antigen lipids from a more inherently sensitive antigen.

These relationships are graphically represented in Chart 1, in which the concentration of heart muscle extractives in two representative antigens is plotted against the sensitiveness of the antigens in the Kahn reaction.* The degree of sensitiveness of standard antigen in the units chosen, is represented by the ordinate of the dotted line ST. AXBCDYE is the sensitiveness-concentration curve for an antigen having a favorable ratio of antigenic to non-antigenic or inhibitory lipids. If, when this antigen is tested, its total concentration of heart muscle lipids is equal to (b) or (c) or (d) units (the concentration corresponding respectively to points B, C and D on the curve) the antigen would show more sensitive reactions than standard. If the concentration of the antigen is equal to (x) or (y) units, corresponding to points X and Y at which the curve intersects the standard sensitiveness line, the antigen would be equal in sensitiveness to standard antigen. In other words,

*A precise definition of the units of concentration and sensitiveness chosen is not essential to an understanding of the problem. In general the concentration may be expressed as weight of heart muscle extractives in unit volume of antigen, and the sensitiveness in terms of Kahn units, all the tests being carried out with a single lot of individual or of pooled syphilitic serum.

there are two concentrations at which an antigen of this type will correspond in sensitiveness to standard. At the concentrations (a) and (e) the antigen will be undersensitive.

This curve immediately suggests methods of correction for an antigen of this type. If the antigen is oversensitive [the initial concentration of the antigen being represented by (b), (c) or (d)], the concentration may be for purposes of correction either decreased to (x) by dilution of the cholesterolized antigen* with cholesterolized alcohol, or may be increased to (y) by the addition of some lipid residue from the same antigen. If the antigen proves to be undersensitive, the method of correction must be selected by trial and error to suit the particular case; correction from the point A to X (or to Y) can take place only by an increase in concentration [as from (a) to (x)]; correction from the point E to Y (or to X) can take place only by decreasing the concentration, that is to say by dilution.

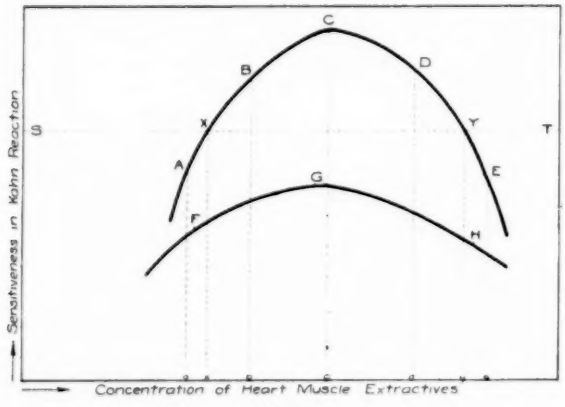

Chart 1.

The curve FGH represents the sensitiveness-concentration relation for an antigen with an unfavorable ratio of antigenic to inhibitory lipids. No matter what its total initial concentration may have been, it cannot be corrected to standard either by increasing or by decreasing the concentration of its own lipids, since its sensitiveness-concentration curve never cuts the standard sensitiveness line ST. The correction of an antigen of this type necessitates the addition of lipids from some other source, as for example from sensitizing reagent.[4]

The practical conclusions that may be drawn from these curves may be summarized as follows:

If an antigen is initially *oversensitive,* it may be corrected either by (a) diluting it with cholesterolized alcohol or by (b) increasing the concentration of its own lipids. In general the former method is to be recommended as simpler and more economical. Experience with a large number of antigens received for standardization at this laboratory has indicated, however, that the

*The concentration referred to in this discussion and in the chart is that of heart muscle extractives, and does not include the constant 0.6 per cent of cholesterol, which is always added to antigens used in the Kahn test.

sensitiveness of many oversensitive antigens is not readily decreased by a moderate amount of dilution (representing a decrease in lipid concentration of from 10 to 25 per cent), but may on the other hand, be decreased to standard by from 10 to 25 per cent increase in concentration. In such cases, in which we are obviously dealing with the CY portion of the AXBCDYE curve, the method of concentration is to be recommended, since that method of correction is always chosen which embodies the least modification of the antigen. Some oversensitive antigens may also be corrected by (c) increasing the amount of salt solution used in preparing the antigen suspension, beyond the requirement of the titer.

If the antigen is *undersensitive*, the deficiency in sensitiveness may be due to an excess of lipids even with a favorable ratio (point E on curve AXBCDYE). In this case the antigen may be corrected by dilution with cholesterolized alcohol until the concentration and sensitiveness correspond to the point Y. Theoretically an antigen might be undersensitive in spite of a satisfactory ratio of its lipid types, if, as initially prepared, it is too dilute (point A on curve AXBCDYE). In practice, however, it is found that the standard method of preparation rarely or never yields an antigen which before correction combines a favorable lipid ratio with a meager total lipid content. Therefore if an antigen is undersensitive, concentration of its own lipids is never used as a method of correction. There remains the possibility that the antigen is undersensitive due to an unfavorable ratio of its lipid types, as illustrated by curve FGH. In a case of this sort, neither dilution with alcohol on the one hand, nor an increase in the concentration of the same type of lipid on the other hand, will result in an adequate increase in sensitiveness, and the correction must be made by adding to the antigen some sensitizing lipids from a different source. If sensitizing reagent is added, the amount required will in no case exceed about 2 or 3 parts per 100 parts of antigen.[4] Thus, although sensitizing reagent is about twice as concentrated as most antigens, the correction of an antigen by the addition of sensitizing reagent affects the total lipid content of the antigen to a very slight extent. The effect of adding sensitizing reagent to a newly prepared antigen can usually be intensified by diluting with cholesterolized alcohol the mixture of antigen and sensitizing reagent. Therefore if an antigen is undersensitive, (a) the method of dilution is first attempted; should this method prove unsuccessful the antigen is corrected by (b) the addition of sensitizing reagent alone or by (c) the addition of sensitizing reagent and cholesterolized alcohol.

PRACTICAL ASPECTS OF ANTIGEN CORRECTION

Reagents Used in Antigen Correction. Two reagents are used in the correction of antigen, cholesterolized alcohol and sensitizing reagent. The former is prepared by dissolving 0.6 gm. cholesterol in 100 c.c. of 95 per cent alcohol as described in previous publications. A complete account of the preparation of sensitizing reagent is given in a previous article of this series.[4]

Calculations Used in Antigen Correction. 1. Addition of Sensitizing Reagent. The amount of sensitizing reagent added to antigen is expressed in terms of the number of parts of reagent added to 100 parts of uncorrected antigen, or, more briefly, and quite accurately for the low proportions of reagent used for antigen correction, as the per cent of reagent present in the corrected antigen. For example to correct an antigen with 1.5 per cent sensitizing reagent, 1.5 c.c. of this reagent are added to 100 c.c. of antigen.

2. Method of Concentration. In concentrating an antigen with its own lipids, the increase in concentration is also interpreted on a percentage basis. Thus a 25 per cent increase in concentration is brought about by evaporating to dryness 25 c.c. of the antigen (noncholesterolized) and dissolving the residue in 100 c.c. of the cholesterolized antigen, or in general, by dissolving the residue from 0.25 c.c. noncholesterolized antigen in each cubic centimeter of cholesterolized antigen.

A scheme that may be found useful in preliminary tests, when an antigen is to be corrected by the method of concentration of its own heart muscle lipids, is to concentrate, as outlined above, a fraction of the antigen by, let us say, 30 per cent, preparing a sufficient quantity for eight or ten series of ten-serum tests. This fraction may be referred to as Fraction 1. If in one or more series of tests it appears that 30 per cent increase in concentration is excessive (antigen sensitiveness reduced below that of the standard), the remainder of Fraction 1 is used for preparing, by dilution with calculated amounts of cholesterolized alcohol, one or two other fractions of concentrations lower than that of Fraction 1, but respectively greater, by let us say 20 and 10 per cent, than that of the original uncorrected (oversensitive) antigen. Fractions 2 and 3 may then be tested in one or more series of 10 serum tests, and if the results with one of the fractions agree with the standard, a volume of that fraction is prepared from the original antigen by the method of concentration, sufficient for three-tube tests with at least 20 sera, for one-tube tests with at least 100 sera, and for several quantitative tests.* In preparing Fraction 2 (and similarly Fraction 3) from Fraction 1 for the preliminary tests, the amount of alcohol to be added to each cubic centimeter of Fraction 1 may be calculated from the formula:

$$V = \frac{P_{c_1} - P_{c_2}}{100 + P_{c_2}}$$

where V = number of c.c. cholesterolized alcohol to be added to 1 c.c. of Fraction 1, P_{c_1} = per cent increase in concentration of Fraction 1 over the concentration of the original antigen, and P_{c_2} = the per cent increase in concentration (again over the concentration of the original antigen) of each fraction that is to be prepared from Fraction 1. For example, to prepare two fractions (2 and 3) respectively 20 and 10 per cent more concentrated than the original, from 8 c.c. of a fraction (Fraction 1) that is 30 per cent more

*In this laboratory antigens are not reported as standard until they have shown comparable results with the standard in several 10-serum series of 3-tube tests, in 1-tube tests with at least 100 sera, and in quantitative tests with two or more sera.

concentrated than the original, we should proceed as follows: For the preparation of Fraction 2 we must add $\left(\frac{30 - 20}{100 + 20}\right) = \frac{1}{12} = 0.083$ c.c. cholesterolized alcohol to each c.c. of Fraction 1, or 4×0.083 c.c. $= 0.33$ c.c. cholesterolized alcohol to 4 c.c. of Fraction 1. Similarly Fraction 3 is prepared by adding $\left(4 \times \frac{30 - 10}{100 + 10}\right) = 0.72$ c.c. cholesterolized alcohol to 4 c.c. of Fraction 1.

3. Method of Dilution. The correction of an antigen by adding cholesterolized alcohol is referred to in terms of a percentage decrease in lipid concentration of the antigen; for brevity in tables, the term percentage dilution is employed, but it must be stressed that this percentage does not coincide in value with the number of parts of alcohol added to 100 parts of antigen. Suppose it is desired to decrease the concentration of the lipids in a given antigen by 20 per cent. If the original concentration was 2 gm. per 100 c.c., the concentration after correction will be 2 minus 20 per cent of 2 or $2 - \left(\frac{20}{100} \times 2\right) = 1.6$ gm. per 100 c.c.

In other words, we must add to the antigen not 20 c.c. alcohol per 100 c.c. of antigen but sufficient alcohol to reduce the lipid concentration of the antigen from 2 gm. per 100 c.c. to 1.6 gm. per 100 c.c. The relation between the desired per cent decrease in concentration and the volume of alcohol to be added is given in Table I. The figures at the right refer to the number of cubic centimeters of cholesterolized alcohol to be added to 1 c.c. antigen, while the column at the left gives the corresponding per cent decrease in the concentration of the antigen, after dilution. To continue our previous illustration, suppose that we wish to reduce the concentration of 45 c.c. antigen by 20 per cent. According to the table we must add to each cubic centimeter of antigen 0.25 c.c. cholesterolized alcohol, and thus to 45 c.c. antigen we add $45 \times 0.25 = 11.25$ c.c. cholesterolized alcohol.

In case it is desired to decrease the concentration of an antigen by a per cent not listed in the table, the following formula may be employed:

$$V = \frac{P_d}{100 - P_d}$$

where P_d = the desired per cent decrease in antigen concentration, and V = the volume of cholesterolized alcohol (in c.c.) to be added to each cubic centimeter of cholesterolized antigen. Suppose it is desired to reduce the concentration of 1,200 c.c. cholesterolized antigen by 27 per cent. In this case $P_d = 27$, therefore $V = \frac{27}{100 - 27} = \frac{27}{73} = 0.37$ c.c. cholesterolized alcohol. Thus, to 1,200 c.c. cholesterolized antigen we must add $1{,}200 \times 0.37 = 444$ c.c. cholesterolized alcohol.

PROCEDURES OF ANTIGEN CORRECTION

When a newly prepared antigen has been titrated according to the method described in previous publications,[2] and has been found to differ from standard antigen in one or more series of comparative 10 serum tests, it is corrected in

the following manner depending on whether it is more sensitive than standard or less sensitive than standard.

1. *Correction of Antigens More Sensitive Than Standard*

(a) *By dilution of the antigen with cholesterolized alcohol.*

(1) Ten c.c. amounts of the more sensitive antigen (cholesterolized) are measured into two 25 c.c. Erlenmeyer flasks.

(2) To one flask is added 1.1 c.c. (10 per cent dilution) and to the other 2.5 c.c. (20 per cent dilution) of cholesterolized alcohol.

(3) The original antigen and the two diluted antigens are tested with 10 sera, most of which are known to give weakly positive reactions, employing standard antigen as a control.

(4) Suppose the antigen which has been modified by 20 per cent dilution gives the same results with these sera as standard antigen; this diluted antigen is given a check test with 10 different sera. If the check series shows comparable results, the entire amount of more sensitive antigen is accordingly corrected by 20 per dilution with cholesterolized alcohol. (For relative volume of alcohol to be added see Table I.) The diluted antigen is once more compared with the standard in one or more series of tests,* and if comparable results are again obtained, the corrected antigen is declared standard.

(5) If neither 10 nor 20 per cent alcohol dilution brings the antigen to standard sensitiveness, but approximates this sensitiveness, different per cent dilutions are tried; if the results are markedly more sensitive than standard even at 25 per cent dilution, then the concentration method is to be used.

TABLE I

DECREASING THE CONCENTRATION OF ANTIGEN BY ADDITION OF CHOLESTEROLIZED ALCOHOL

P_d DESIRED PER CENT DECREASE IN CONCENTRATION (OR PER CENT DILUTION)	V VOLUME, IN C.C. OF CHOLESTEROLIZED ALCOHOL TO ADD TO 1 C.C. ANTIGEN
5	0.05
6	0.06
*7	0.075
8	0.087
9	0.10
*10	0.11
12	0.14
*15	0.18
18	0.22
*20	0.25
25	0.33
30	0.43
33	0.50
35	0.54
40	0.67
45	0.82
50	1.0

*These per cent dilutions are the most frequently employed in antigen correction.

*See footnote on page 984.

Table II illustrates the correction of a more sensitive antigen (Antigen I) by dilution with cholesterolized alcohol. In this case the antigen after 30 per cent dilution with cholesterolized alcohol gave results comparable to the standard.

(b) *By concentration of the lipids in the antigen.*

(1) Into 2 small evaporating dishes are measured, respectively, 1 and 2 c.c. amounts of the oversensitive antigen in a noncholesterolized state.

(2) With the aid of an electric fan, the two amounts are evaporated to dryness. This takes but a few minutes.

(3) Each of the residues is dissolved in a 10 c.c. amount of cholesterolized antigen; the modified antigens thus formed contain respectively 10 and 20 per cent more extract lipids than the original antigen.

(4) The original antigen and the two modified antigens are tested with 10 sera employing standard antigen as a control.

(5) If the modified antigen in which the increase in concentration of lipids is 20 per cent, gives the same results with sera as does standard antigen, the check examinations are repeated with 10 additional sera (to eliminate the possibilities of error) and the entire amount of more sensitive antigen is accordingly concentrated to the extent of 20 per cent. The newly corrected antigen is finally declared standard after a given number of additional comparative tests with standard antigen, similar to those described above in the case of correction by dilution.

TABLE II

A MORE SENSITIVE ANTIGEN (ANTIGEN I) CORRECTED TO STANDARD REQUIREMENTS BY DILUTION WITH CHOLESTEROLIZED ALCOHOL

SERUM NO.	STANDARD ANTIGEN TITER 1 + 1.1			ANTIGEN I								
				UNCORRECTED TITER 1 + 1.65			15% DILUTION WITH CHOLESTEROLIZED ALCOHOL TITER 1 + 1.3			30% DILUTION WITH CHOLESTEROLIZED ALCOHOL TITER 1 + 1.1		
	1	2	3	1	2	3	1	2	3	1	2	3
1.	−	−	−	−	−	−	−	−	−	−	−	−
2.	−	−	±	±	++	+++	−	±	+++	−	−	±
3.	−	+	++	+	+++	+++	±	++	+++	−	+	++
4.	−	−	−	±	±	++	−	±	±	−	−	−
5.	−	±	±	±	++	+++	−	±	+	−	±	±
6.	−	−	±	−	±	+++	±	±	++	−	±	±
7.	−	±	±	±	++	+++	±	+	+	−	±	±
8.	−	+	+	+	+++	+++	±	++	+++	−	+	+
9.	−	−	±	±	±	++	−	±	+	−	−	±
10.	−	−	−	−	−	±	−	−	±	−	−	−

(6) If neither 10 nor 20 per cent increase in concentration brings the antigen to standard sensitiveness, the results obtained with these concentrations will indicate other concentrations which should be tried.

Table III illustrates the correction of a more sensitive antigen (Antigen I) to standard requirements by increase in concentration. In the case of this antigen, a 25 per cent increase in its lipid concentration was necessary for

correction. An interesting observation regarding this antigen is that its titer before correction was 1 + 1.65, while its titer after correction was 1 + 1.3.

It should be noted that Antigen I, corrected by an increase in its lipid concentration (Table III) is the same antigen that was corrected by dilution with alcohol (Table II). The possibility of correcting an oversensitive antigen by two seemingly opposite alternative methods is to be expected from the nature of the relationship between the lipid concentration of an antigen and its sensitiveness. As was indicated in the first part of this article, antigen sensitiveness is reduced by increasing as well as by decreasing its lipid concentration.

TABLE III

A MORE SENSITIVE ANTIGEN (ANTIGEN I) CORRECTED TO STANDARD REQUIREMENTS BY INCREASE IN LIPID CONCENTRATION

SERUM NO.	STANDARD ANTIGEN TITER 1 + 1.1			ANTIGEN I								
				UNCORRECTED TITER 1 + 1.65			LIPID CONCENTRATION INCREASED 50% TITER 1 + 1.3			LIPID CONCENTRATION INCREASED 25% TITER 1 + 1.3		
	1	2	3	1	2	3	1	2	3	1	2	3
1.	−	−	−	−	−	−	−	−	−	−	−	−
2.	−	±	+	±	+	++	−	−	−	−	±	+
3.	±	+	++	++	+++	+++	−	−	±	−	+	++
4.	−	±	±	±	+	+++	−	−	±	−	±	±
5.	−	±	±	±	++	+++	−	−	±	−	±	+
6.	−	±	±	±	++	+++	−	−	−	−	±	±
7.	−	±	+	+	++	+++	−	±	±	−	±	+
8.	−	±	++	++	+++	++++	−	−	−	±	±	++
9.	−	−	±	±	+	++	−	−	−	−	−	±
10.	−	−	−	−	−	±	−	−	−	−	−	−

(c) *By increasing the amount of salt solution in the antigen suspension beyond requirement of titer.*

(1) We shall assume that the titer of the oversensitive antigen is 1 + 1.3. Three antigen suspensions are prepared by mixing 1 c.c. amounts of this antigen with 1.3, 1.5, and 1.7 c.c. quantities of salt solution.

(2) These three suspensions are tested with sera, employing a suspension prepared from standard antigen as a control. If the suspension prepared with 1.5 c.c. salt solution gives results with sera of the same degree of sensitiveness as that of standard antigen, this amount of salt solution (instead of the amount indicated by the titer) might be employed in the preparation of the suspension from this antigen. In this case it is the antigen suspension and not the antigen itself that is modified, in order to reduce the sensitiveness to standard requirements.

As in the case of the previous methods of antigen correction, this method also requires a large number of comparative tests with standard antigen before the antigen with the modified titer may be declared as standard. Table IV illustrates the correction of a more sensitive antigen (Antigen II) by increasing beyond the titer the amount of salt solution in the antigen suspension.

TABLE IV

A MORE SENSITIVE ANTIGEN (ANTIGEN II) CORRECTED TO STANDARD REQUIREMENTS BY INCREASING BEYOND THE TITER THE AMOUNT OF PHYSIOLOGIC SALT SOLUTION IN THE ANTIGEN SUSPENSION

SERUM NO.	STANDARD ANTIGEN TITER 1 + 1.1			ANTIGEN II								
				UNCORRECTED TITER 1 + 1.5			TITER MODIFIED TO 1 + 1.7			TITER MODIFIED TO 1 + 1.9		
	1	2	3	1	2	3	1	2	3	1	2	3
1.	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
2.	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
3.	−	±	++	±	+	+++	−	±	++	−	−	−
4.	−	±	+	−	±	+++	−	±	+	−	−	−
5.	−	±	+	−	±	++	−	±	+	−	−	−
6.	−	±	±	−	±	++	−	−	±	−	−	−
7.	−	−	±	−	−	+	−	−	±	−	−	−
8.	−	−	±	−	−	±	−	−	−	−	−	−
9.	−	−	−	−	−	±	−	−	−	−	−	−
10	−	−	−	−	−	−	−	−	−	−	−	−

TABLE V

A LESS SENSITIVE ANTIGEN (ANTIGEN III) CORRECTED TO STANDARD REQUIREMENTS BY DILUTION WITH CHOLESTEROLIZED ALCOHOL

SERUM NO.	STANDARD ANTIGEN TITER 1+1.1			ANTIGEN III								
				UNCORRECTED TITER 1+1.3			13 PER CENT DILUTION WITH CHOLESTEROLIZED ALCOHOL TITER 1+1.1			23 PER CENT DILUTION WITH CHOLESTEROLIZED ALCOHOL TITER 1+1.1		
	1	2	3	1	2	3	1	2	3	1	2	3
1.	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
2.	−	+++	++++	−	+	++++	−	++	++++	−	+	++++
3.	−	++	++++	−	±	++++	−	++	++++	−	+	++++
4.	−	++	+++	−	±	++	−	++	++++	−	+	++
5.	−	+	++++	−	±	++	−	++	++++	−	+	+++
6.	−	+	++++	−	±	+++	−	++	+++	−	+	++
7.	−	+	+++	−	−	++	−	+	+++	−	±	++
8.	−	±	+++	−	±	+	−	+	+++	−	±	++
9.	−	±	+	−	−	±	−	±	+	−	−	−
10.	−	−	−	−	−	−	−	−	−	−	−	−

TABLE VI

AN UNDERSENSITIVE ANTIGEN (ANTIGEN IV) CORRECTED TO STANDARD REQUIREMENTS BY ADDITION OF SENSITIZING REAGENT

SERUM NO.	STANDARD ANTIGEN TITER 1+1.1			ANTIGEN IV								
				UNCORRECTED TITER 1+1.0			ADDITION OF 0.5 PER CENT SENSITIZING REAGENT TITER 1+1.0			ADDITION OF 1.5 PER CENT SENSITIZING REAGENT TITER 1+1.1		
	1	2	3	1	2	3	1	2	3	1	2	3
1.	−	−	−	−	−	−	−	−	−	−	−	−
2.	−	+	++	−	−	±	−	±	±	−	+	++
3.	−	±	+	−	−	±	−	−	±	−	±	+
4.	−	−	+	−	−	±	−	−	±	−	±	+
5.	−	±	+	−	−	±	−	−	±	−	+	++
6.	−	±	+	−	−	±	−	−	±	−	±	+
7.	−	±	±	−	−	±	−	±	±	−	±	++
8.	++	+++	+++	±	+	+	±	+	+	++	+++	+++
9.	−	±	+	−	−	±	−	−	±	−	±	+
10.	−	−	−	−	−	−	−	−	−	−	−	−

TABLE VII

A LESS SENSITIVE ANTIGEN (ANTIGEN V) CORRECTED TO STANDARD REQUIREMENTS BY ADDITION OF SENSITIZING REAGENT PLUS DILUTION WITH CHOLESTEROLIZED ALCOHOL

SERUM NO.	STANDARD ANTIGEN TITER 1 + 1.1			ANTIGEN V								
				UNCORRECTED TITER 1 + 1.0			ADDITION OF 0.5 PER CENT SENSITIZING REAGENT PLUS 20 PER CENT DILUTION WITH CHOLESTEROLIZED ALCOHOL TITER 1 + 1.0			ADDITION OF 1.0 PER CENT SENSITIZING REAGENT PLUS 20 PER CENT DILUTION WITH CHOLESTEROLIZED ALCOHOL TITER 1 + 1.1		
	1	2	3	1	2	3	1	2	3	1	2	3
1.	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
2.	++++	++++	++++	±	++++	++++	++++	++++	++++	++++	++++	++++
3.	+++	++++	++++	−	+++	++++	++	++++	++++	++	++++	++++
4.	++	++++	++++	−	+	+++	+	++++	++++	++	++++	++++
5.	+	++++	++++	−	+++	++++	+	++++	++++	+	++++	++++
6.	±	+++	++++	−	−	++	−	+++	++++	±	++++	++++
7.	±	+++	++++	−	−	±	±	++	+++	−	+++	++++
8.	−	+	++++	−	−	−	−	±	++++	−	±	++++
9.	−	±	+++	−	−	−	−	±	+++	−	±	+++
10.	−	−	−	−	−	−	−	−	−	−	−	−

2. *Correction of Antigens Less Sensitive Than Standard*

(a) *By dilution of the antigen with cholesterolized alcohol.*

Since antigens may be of low sensitiveness due to excessive concentration of lipids obviously it should be possible to correct certain undersensitive antigens by dilution with cholesterolized alcohol. This method of correction which is carried out as was described for an oversensitive antigen, method (a), is illustrated in Table V (Antigen III).

(b) *By addition to the antigen of sensitizing reagent.*

(1) Ten c.c. amounts of the undersensitive antigen are measured into two 25 c.c. Erlenmeyer flasks.

(2) To one flask is added 0.05 c.c. sensitizing reagent while to the other is added 0.1 c.c. sensitizing reagent.

(3) The two modified antigens are tested with sera in the usual manner, employing standard antigen as a control. If one of the modified antigens gives results comparable with standard, the whole amount of antigen is corrected accordingly, and after repeated check tests, is declared standard. In case no parallel results are obtained, other combinations of sensitizing reagent and antigen are tried. It should be noted that the sensitiveness of an antigen is usually increased very markedly when from 2 to 5 parts of sensitizing reagent are added to 100 parts of antigen. When the proportion of sensitizing reagent used exceeds 6 or 7 parts per 100, the precipitates in the serum reaction become extremely fine, and with a sufficient excess of sensitizing reagent, the precipitation reaction with syphilitic sera will be completely inhibited.

Table VI illustrates the correction of an undersensitive antigen (Antigen IV) by the addition of sensitizing reagent.

(c) *By addition to the antigen of sensitizing reagent plus dilution with cholesterolized antigen.*

(1) Ten c.c. amounts of the undersensitive antigen are measured into two 25 c.c. Erlenmeyer flasks.

(2) To one flask are added 0.05 c.c. sensitizing reagent (0.5 per cent) and 1.1 c.c. cholesterolized alcohol (10 per cent dilution) while to the other are added 0.1 c.c. sensitizing reagent (1.0 per cent) and 2.5 c.c. cholesterolized alcohol (20 per cent dilution).

(3) The two modified antigens are tested in the usual way, with standard antigen as a control. In case neither of the modified antigens corresponds in sensitiveness to the standard, other combinations of sensitizing reagent and cholesterolized antigen are employed.

Tabel VII illustrates the correction of an undersensitive antigen (Antigen V) by the combined use of sensitizing reagent and cholesterolized alcohol.

3. *Summary of Methods of Antigen Correction*

The following outline summarizes the methods of antigen correction.

Types of Uncorrected Kahn Antigens Showing Variations in Sensitiveness	*Methods Employed in Correction*
I. Antigen Comparable in Sensitiveness to Standard Antigen	Correction unnecessary.

II. Antigen More Sensitive Than Standard Antigen	(a) Dilution with cholesterolized alcohol. (b) Concentration of lipids. (c) Increasing amount of salt solution in antigen suspension beyond requirement of titer.
III. Antigen Less Sensitive Than Standard Antigen	(a) Dilution with cholesterolized alcohol. (b) Addition of sensitizing reagent. (c) Addition of sensitizing reagent plus dilution with cholesterolized alcohol.

4. *Preferred Methods of Correction*

(a) We believe it is desirable to employ as far as possible a standard titer for antigens. In this laboratory, all antigens used in the 3-tube Kahn test during the past five years have been corrected to a standard titer of 1 + 1.1. We see no objection, however, to the employment of closely similar titers, such as 1 + 1.2 or 1 + 1.3. Furthermore, if an antigen can be corrected by a small increase (0.1 or 0.2 c.c.) in the salt solution used in preparing the suspension beyond the amount indicated by the titer, this method should be found satisfactory. It should be emphasized that in the correction of antigen to standard sensitiveness, we have not found it necessary in most cases to retitrate the modified antigens before testing with sera.*

(b) It is of interest to note that an antigen more sensitive than standard usually requires a comparatively large amount of salt solution in the titer. The preferred methods of correcting such antigens tend to reduce the amount of salt solution in the titer and at the same time decrease the sensitiveness of the antigen. One of these methods involves the dilution of the antigen with cholesterolized alcohol, while the other involves the concentration of the antigen with its own lipids. The choice between these two methods is made as follows: Dilution of the more sensitive antigen with cholesterolized antigen is tried first. If the antigen can be corrected with an amount of cholesterolized alcohol not exceeding 20 per cent, this is the method to be adopted; if the amount of alcohol required for correction exceeds 20 per cent, the concentration method is preferred. Experience with highly sensitive antigens further indicates that those requiring excessive amounts of alcohol dilution for correction to standard sensitiveness, usually require but a small amount of lipid concentration for similar correction.

It occasionally happens that the entire amount of oversensitive antigen has been cholesterolized before standardization. When noncholesterolized antigen is not available, it is obviously impossible to concentrate the antigen with its own lipids without thereby increasing the concentration of cholesterol be-

*Correction with sensitizing reagent may result in an increase in titer.

yond its proper value of 0.6 per cent. In that case, one may add to the antigen in question the lipid residue from another noncholesterolized Kahn antigen.

(c) Turning to the methods of correction of antigens less sensitive than standard, the titer of the antigens gives an indication as to which method to employ. An undersensitive antigen having a titer which exceeds 1 + 1.1 (1 +1.2, 1 + 1.3, etc.), indicating high concentration of lipids, is best corrected by dilution with cholesterolized alcohol. An undersensitive antigen which has a titer of 1 + 1, 1 + 0.9, 0.8 or 0.7, indicating meager concentration of lipids, is most suitably corrected by adding sensitizing reagent and cholesterolized alcohol. Our experience indicates that antigens having titers of 1 + 1.1 are in practically all cases closely similar in sensitiveness to standard antigen. Should such antigens require correction, it will usually be found that dilution with cholesterolized alcohol is a suitable method. It should be added that the combined use of sensitizing reagent and cholesterolized alcohol in correcting undersensitive antigens has given better results in our hands than the use of sensitizing reagent alone.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

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ANEROBES: A Simplified Method for the Cultivation of Anerobes in Fluid Media, Petroff, S. A. Arch. Path. 5: 834, 1928.

Five or six holes 2 mm. in diameter are made on the lower end of a tube that is from 10 to 12 cm. long and 2 cm. in diameter. The tube is well packed about one-third with cotton. A rubber tubing with an inside diameter of 20 mm., an outside diameter of from 25 to 26 mm. long is slipped over the absorption tube. The thickness of this tube will depend on the size of the mouth of the culture flask. The tube is then wrapped in paper in the usual way, sterilized at 15 pounds pressure and kept until needed. The broth media is inoculated as usual. The cotton plug of the flask is aseptically replaced with the absorption tube. Pyrogallol is placed in the tube on top of the cotton packing. Ten per cent sodium hydroxide is then slowly poured into the tube over the pyrogallic acid until the cotton packing is well dampened. An excess must be avoided. The absorption tube is then closed with a rubber stopper and the connections well sealed with paraffin.

GRAM-STAIN: A Further Modification, Kopeloff, N. and Cohen, P. Stain Tech. 3: No. 2, 64, 1928.

Air-dry film and fix with least amount of heat necessary.

Flood with dye for five minutes. Previously mix 30 drops of a 1 per cent aqueous solution of crystal violet or methyl violet 6B with 8 drops of a 5 per cent solution of sodium bicarbonate. Allow the mixture to remain for five minutes or more.

Flush with iodine solution for two minutes. (Two grams iodine dissolved in 10 c.c. normal sodium hydroxide solution and 90 c.c. water added.)

Drain without blotting but do not allow film to dry.

Add a mixture of equal parts of acetone and alcohol drop by drop until the drippings are colorless (10 seconds or less).

Air-dry slide.

Counterstain for twenty seconds with 0.1 per cent aqueous solution of basic fuchsin.

Wash off excess stain by short exposure to tap water and air-dry. If slide is not clear immersion in xylol is recommended.

GLUCOSE SOLUTIONS: The Prevention of Reactions Following Intravenous Injections of Glucose Solution, Titus, P., and Dodds, P. Am. J. Obst. & Gynec. 14: No. 2, 181, 1927.

The average dose for an adult is about one gram per kg. of body weight. An average adult weighs between 50 and 75 kg. so 75 gm. is the initial dose, subsequent doses to be 50 gm. There will be some spill through the kidneys, but the appearance of sugar in the urine following such an injection is of no especial significance.

The glucose should be chemically pure. Several different preparations have been used and while there is little choice if chemically pure the authors have usually used Merck's c.p. anhydrous glucose.

The glucose should be dissolved in *freshly* double distilled, uncontaminated water, and not in salt solution of sodium bicarbonate solution as has been variously recommended. In the preparation of a single dose, 50 gm. of the glucose is carefully weighed and then dissolved in 200 c.c. of freshly distilled water, using only glassware which has been thoroughly washed in distilled water. Before preparing for sterilization the solution should be filtered

at least five or six times to remove even tiny particles of dust or cotton fibers. For the sake of simplicity in making any necessary calculations for dilutions it is convenient to have each flask contain 25 gm. of glucose in 100 c.c. of water (25 per cent) of a "half-dose."

The flasks of glucose solution after being properly stoppered with cotton plugs in gauze and the top sealed with lead foil, should be sterilized in a steam sterilizer for one-half hour at 100° C. on three successive days. It may be prepared more quickly for emergencies by being sterilized in an autoclave at 15 pounds pressure for twenty minutes.

In either case sufficient space should be left between the level of the solution in the flask and the stopper so that the solution will not be forced by vacuum up against the stopper. This is also a precautionary measure against the possibility of splashing the solution against the stopper in any handling of the flasks.

Solutions showing caramelization or sediment after sterilization should be discarded.

Glucose solution kept in flasks stoppered with cotton, or cotton and gauze, and sealed with lead foil will show comparatively little change in the hydrogen-ion content over a considerable period of time, even though buffer salts have not been added, so that solutions may be prepared in advance of the need for them.

Preferably solutions should not be kept longer than four weeks unless hermetically sealed, and the solution is probably safer when it is fresh. It is seldom, however, that a reaction can be traced to a solution that is not more than from two to four weeks old.

Ampules of glucose (dextrose) solution are now on the market and apparently have been carefully and properly prepared by their manufacturers. Many of these have been used with satisfactory results, and the only one open to particular comment is that of one firm to which cresol has been unnecessarily added as a preservative. This ampule contains only 10 gm. of glucose so that if enough ampules are used to obtain a therapeutic dose for anything other than insulin hypoglycemia, a reaction is a common aftermath on account of the total amount of cresol injected.

The injection should proceed slowly at the rate of 4 c.c. per minute for a 25 per cent solution, and during this period the solution should be kept hot. The solution is ordinarily injected from a salvarsan tube, using a small caliber needle, and having the tube coiled in a basin of hot water to keep the temperature of the flowing fluid up to from 100° to 110° F.

Fresh rubber tubing should not be used until after it has been thoroughly washed in running water, then boiled in clean water (without sodium carbonate), again washed, and finally sterilized in the autoclave. Chemical contamination from new rubber tubing has been suggested as a cause of reactions following these injections.

The salvarsan tube and the connecting tubes, as well as the rubber tubing should be thoroughly cleansed and then sterilized in an autoclave, because when merely boiled, sediment from the water is often to be seen within the glass. Before beginning the injection the system should be rinsed out by running distilled water through the salvarsan tube, the rubber tubing, the connections, and the needle.

It is recommended that new rubber tubing should be treated before using as follows: (1) Soak in soap and water one hour. (2) Wash well with soap and water. (3) Wash in running water. (4) Soak for six hours in 4 per cent solution of sodium hydroxide. (5) Wash well in running water. (6) Wash well in distilled water.

COLLOIDAL GOLD: A Method of Preparation, Leiboff, S. L. Arch. Dermat. & Syph. **17:** 380, 1928.

Clean the glassware by boiling for half an hour in a solution of ivory soap; wash well with tap water and then soak for a few hours in warm chromic acid cleaning solution. This solution is prepared as follows:

Potassium dichromate	80 gm.
Concentrated sulphuric acid	120 c.c.
Water	1000 c.c.

Wash well with tap water and lastly with distilled water and dry in a hot oven.

Prepare cresol phthalein indicator paper by immersing strips of fat-free ashless filter paper into a 1 per cent alcoholic solution of cresol phthalein (1 gm. of cresol phthalein in 100 c.c. of 95 per cent alcohol), and dry at room temperature.

In a large clean beaker place 1,000 c.c. of ordinary distilled water and 10 c.c. of a 1 per cent solution of gold chloride in water. Add 1 gm. of saccharose (cane sugar) and dissolve by mixing with a glass stirring rod. Add slowly, drop by drop, tenth-normal sodium hydroxide, mixing the contents all the while. From time to time, take out one drop of the solution on the end of the stirring rod and place on a piece of the cresol phthalein indicator paper; when the end-point is reached, the spot of the paper touched by the solution turns pink. A good pink color should be obtained, which should not fade in thirty seconds. It takes about 5 c.c. of the sodium hydroxide solution to produce the proper reaction. Place the beaker over a Bunsen flame and heat; before the boiling point is reached a faint color will develop, and when the solution begins to boil a beautiful clear salmon red gold solution will be obtained. Boil until no more color develops. It is better to boil it a little too long rather than not long enough. When cool, titrate the solution as follows:

Into each of three test tubes place 5 c.c. of colloidal gold and add to the first tube 0.4 c.c. of a 1 per cent solution of sodium chloride; to the second tube add 1.7 c.c. of 1 per cent solution of sodium chloride; the third tube serves as a control. Let it stand for one hour at room temperature and follow one of the two following procedures:

If the first tube remains unchanged and the second tube is not completely reduced, the solution is too alkaline and, accordingly, not sensitive enough. Set up seven tubes containing 5 c.c. of the colloidal gold each, and titrate as shown in the table.

The tube containing the least amount of acid which remained unchanged is taken as correct, and the amount of acid to be added is calculated from this.

If the first tube in the preliminary titration shows a slight reduction and the second tube is completely reduced the gold sol is slightly too acid and, hence, too sensitive. Set up seven tubes as indicated in the first procedure but add the corresponding amounts of hundredth-normal sodium hydroxide instead of the acid.

TITRATION OF COLLOIDAL GOLD

TUBE	1	2	3	4	5	6	7 (control)
Number of c.c. of colloidal gold	5	5	5	5	5	5	5
Number of c.c. of hundredth-normal hydrochloric acid	0.1	0.2	0.3	0.4	0.5	0.6	none
Number of c.c. of 1 per cent sodium chloride	0.4	0.4	0.4	0.4	0.4	0.4	0.4

A number of colloidal gold solutions prepared by this method were tested on known paretic, syphilitic and normal spinal fluids, and were checked by a colloidal gold sol prepared with triply distilled water by the formaldehyde process. Good agreements were obtained, and in no instance was a reaction obtained with normal spinal fluids.

LIVER FUNCTION TESTS: Practical Value of Liver Function Tests, Piersol, G. M. and Rothman, M. M. J. A. M. A. **91**: 1768, 1928.

Of all the liver function tests thus far devised, those of greatest clinical value and general usefulness are (1) the estimation of urobilinogen; (2) the determination of serum bilirubin (either by the quantitative van den Bergh test or, better, by the icterus index), and (3) the estimation of the degree of retention of the dye bromsulphthalein.

Urobilinogen is probably the most delicate single test for liver dysfunction. It is always increased, even when the injury to the parenchyma is slight or when excessive blood destruction brings about an increase in bile pigment formation. In the authors' experience, urobilinogen is constantly increased to a noteworthy degree in portal cirrhosis. Slight increases in urobilinogen have been noted from time to time in a limited number of patients in whom liver disorder was suspected, but not evident clinically.

In many conditions a persistent increase of urobilinogen was indicative of a residual hepatitis.

The presence of latent icterus, as revealed by serum bilirubin estimations, is of distinct importance both diagnostically and prognostically, because in this way even slight degrees of bile retention may be determined.

The retention of bromsulphthalein is valuable confirmatory evidence of liver dysfunction. Such retention rarely occurs unless one or both of the liver function tests already mentioned are positive, with the exception of portal cirrhosis in which dye retention occurred in the absence of hyperbilirubinemia. In these authors' experience, 2 mg. per kilogram of body weight of this dye is as accurate as the larger 5 mg. dose.

ACETONE BODIES: Citric Acid and Citrates as a Source of Error in the Van Slyke Method, Clarke, B. E. and Hausmann, G. H. Arch. Path. 6: No. 5, 881, 1928.

The authors demonstrate that sodium citrate used as an anticoagulant is a source of error in quantitative determination of acetone body content in blood by the method of Van Slyke. Citric acid administered by mouth does not have any effect on either the acetone body content or the power of the blood to combine with carbon dioxide. Citric acid and its salts are not a source of error in quantitative determination of acetone body content in urine by this method.

Combined Nuclear and Differential Stain, Brilmyer, G. J. Science 68: 114, 1928.

A new method for combining and manipulating Delafield's hematoxylin and Mallory's connective tissue stain is as follows:

(1) Stain in Delafield's hematoxylin, five minutes, (2) wash to remove excess of stain, (3) stain in 0.2 per cent aq. solution of acid fuchsin one minute, (4) wash to remove excess of stain, (5) stain in the following solution two to three hours: Aniline blue (water solution) 0.5 gm., Orange C 2.0, and 1 per cent phosphomolybdic acid 100 c.c., (6) wash to remove excess of stain, (7) pass successively and rapidly through 35, 75, and 95 per cent alcohol, (8) dehydrate in absolute alcohol thirty to sixty seconds (anhyd. acetone may be used in place of absolute alcohol), (9) clear in xylene, (10) mount with cover glass. With this procedure nuclei appear a rich red, epithelial cells pink, connective tissue blue and muscle red. Red blood corpuscles stain yellowish in veins, reddish in arteries. Colloid and mucus stain blue. Sections stained by this method have not faded in five years.

MALARIA: Demonstration of Leishman-Donovan Bodies and Malarial Parasites in Venous Blood, Mukherjee, H. N. Calcutta M. J. 22: No. 9, 487, 1928.

The fact that the corpuscles in blood from kala-azar and malaria cases sediment rapidly may be utilized for the detection of malarial parasites or Leishmania. About 1 c.c. of citrated or oxalated blood taken from a vein is allowed to stand in long serologic pipette tubes for three to four hours. With a fine capillary pipette a little of the clear plasma just above the red blood cell deposit, together with about 1 mm. of the topmost layer of red blood cells is removed and placed on a clean slide. A film is made and stained in the usual way. In sedimentation the uninfected red cells are at the bottom, those with malarial parasites next and the leucocytes in which Leishmania may occur above these.

SPORES: Wright's as a Differential Spore Stain, Dutton, L. O. Stain Technic 3: No. 4, 144, 1928.

A heavy suspension of the organism to be stained is made in 0.4 c.c. of distilled water in a fairly large test tube (150 by 13 mm.). To this suspension 0.1 c.c. of Wright's staining solution (50 to 100 mg. to 60 c.c. of methyl alcohol) is added. The tube is then tightly stoppered to prevent evaporation of the alcohol and immersed in boiling water for about ten minutes. It is removed from the water-bath and allowed to cool for about half a minute to prevent the contents from bubbling out when it is opened. Loopfuls of the stained organism

are then spread on slides and dried. Examination shows the cytoplasm of the sporangium stained a reddish brown and the spore bodies a fairly intense blue.

If desired, a loopful of the organism may be mixed with an equal quantity of saturated aqueous solution of nigrosin, as in the Dorner technic, and then spread on slides. The spores are then more plainly seen. Nigrosin weaker than saturated solution may be used with the Wright's stain, for it is a very powerful decolorizing agent for the red in the cytoplasm. As high a dilution as 1 to 100 renders the bacterial cell, except the spore, completely colorless.

SCHISTOSOMIASIS: A Precipitin Test. Taliagerro, W. H., Hoffman, W. A., and Cook, D. A. J. Prevent. Med. 2: No. 5, 395, 1928.

1. Aqueous extracts made by extracting dried livers of Planorbis guadeloupensis containing larval stages of Schistosoma mansoni (1 c.c. of extractive to 0.025 gm. powder) with the slightly alkaline solution of coca, saline or phenol, yield specific test antigens. Seventy-seven precipitin tests on the serums of twenty-eight persons with stools positive for S. mansoni gave 63 positive (29 +++, 24 ++ and 10 +) and 14 negative tests; 25 tests on five persons known to be negative for both S. mansoni and syphilis gave one positive (+) and 23 negative tests; 17 tests on four persons negative for S. mansoni but positive for syphilis gave 9 positive (2 ++ and 7 +) and 8 negative. The active principle in these aqueous extracts is precipitated with the albumin fraction, i.e., it is not precipitated with half saturation with ammonium sulphate, but is precipitated with entire saturation.

2. Attempts to prepare in vitro antigens by extracting the dried cercarial snail livers with N/20 or N/10 hydrochloric acid and N/20 or N/10 sodium hydroxide and adjusting the supernatant to P_H 7.4 were all unsuccessful.

3. When antigens are prepared by a thorough extraction of the dried cercarial powder in a Soxhlet apparatus with ether, absolute alcohol or both, followed by the extraction of the lipoid-free residue with coca's solution (containing 0.05 per cent $NaHCO_3$), and the use of the clear supernatant (P_H about 7.4 without further adjustment), the following advantages are found over the antigens noted in Section 1.

(a) Proteins and the reactive material go into solution much more rapidly so that antigens can be prepared in twenty minutes and all the antigens of each set are uniform.

(b) The reactions with schistosomal serums are much stronger. Thus, the following results were obtained with antigens prepared from the same cercarial powder as in Number 1, but after lipoid removal; 20 tests on eight known schistosomal cases were all positive (18 +++ and 2 ++) whereas 20 tests on four nonschistosomal and nonsyphilitic serums were all negative.

(c) The cloudiness and spontaneous precipitation sometimes encountered with the antigens prepared as in Section 1 were completely eliminated by alcohol extraction, and largely so by ether extraction.

(d) The pseudopositives in syphilitic serums were not eliminated by extraction with alcohol, ether, or both. Thus, using the same lot of powder as in Section 1, antigens from the lipoid-free powder gave, in 16 tests on four syphilitic cases not infected with S. mansoni, 8 positive tests (3 ++ and 5 +) and 8 negatives.

4. By centrifugation the larval schistosomes can be separated from the "liver" tissue in macerated fresh infected snail "livers." This separation is so complete that the serums of two monkeys immunized against the normal snail "liver" gave a +++ reaction with antigens prepared from uninfected snail livers and whole infected snail livers, but a negative reaction with antigen prepared from the concentrated larval schistosomes. Aqueous extracts, prepared from the lipoid-free powder of these concentrated larvae, are potent antigens and preliminary experiments suggest that they do not give pseudopositives with syphilitic serums.

5. Antigens prepared from lipoid-free powders of normal uninfected snail "livers" give uniformly negative reactions with schistosomal serums.

NEPHRITIS: The Andrewes Diazo Reaction In, Eastland, J. S. and Schmidt, E. G. Arch. Int. Med. **43**: No. 4, 472, 1929.

From a fairly extensive study of this reaction the authors conclude that:

1. The Diazo reaction is a simple test for advanced renal damage which can be performed with the most meager laboratory equipment.

2. A positive test was never found to accompany normal or low elimination of phenolsulphonephthalein.

3. A positive test was always found to be associated with a marked retention of nitrogenous products.

4. The test was found to be of considerable prognostic aid in the cases of advanced or extensive renal damage.

BERI-BERI: The Etiology of, Matsumura, S., et al., J. A. M. A. **92**: No. 16, 1325, 1929.

The authors describe briefly a bacillus isolated from the feces in beriberi, not found in normal individuals, producing the disease in fowls, and agglutinated by the serum from cases of the disease.

An organism resembling B. coli-communior was found, usually in relatively large numbers, among the intestinal types isolated from experimental animals and also from human cases. The microbe ferments saccharose readily; hence Endo plates containing this sugar in place of lactose lend themselves well for isolation.

The organism, like B. coli, is gram-negative and motile and does not form spores. It has peritrichal flagella. It does not liquefy gelatin; it produces indol, coagulates milk and shows a rather distinctive colony on agar plates. It ferments the common hexoses, as well as maltose, lactose, saccharose, xylose, the hexatomic alcohols, mannitol and sorbitol, and glycerin, rhamnose and arabinose, all with the production of gas and acid. Fermentation of dulcitol and salicin is irregular. Inositol, adonitol, erythritol, and a-methyl-glucoside are not fermented. The organism has been named Bacillus beriberi. The differentiation of B. beriberi from B. coli-communior depends on the fact that the beriberi bacillus agglutinates and also gives a complement-fixation reaction; B. coli-communior does not.

TUBAL PREGNANCY: The Pyramidone Test, Klein, S. M. Arch. f. Gynäk. **135**: 256, 1928.

The author reports upon Jegeroff's modification in 68 cases.

To a mixture of 3 c.c. of 5 per cent alcoholic pyramidone solution, 8 drops of 3 per cent hydrogen peroxide and 8 drops of 50 per cent acetic acid, add 1 drop of the serum to be tested. An amethyst blue color indicates the presence of hematin.

A positive result was obtained in 90 per cent of cases of ectopic gestation. Positive results were also encountered in 2 cases of apoplexia ovarii and in 5 cases of intermenses.

Klein believes the test of value in the diagnosis of tubal pregnancy.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Serum Diagnosis by Complement Fixation**

THE life of medical books is, as a rule, rather ephemeral for obvious reasons. This volume, however, it may be predicted will remain a standard text for years to come and, in all probability will never be displaced as a fountainhead of information on this subject.

There are, perhaps, few laboratory procedures which have come into more common use than that commonly, but in many ways improperly, known as the Wassermann test. There are few procedures, also, which have been subjected to more misuse, both in the manner in which it has been applied and the way in which its results have been interpreted and utilized as concerns the patient. For these reasons, and many more which readily come to mind, a clear-cut and authoritative survey of the situation has long been demanded.

Few men have been more closely associated with the serologic study of disease than Dr. Kolmer, none more responsible for the development of modern serologic technic, and none better able to discuss it and its clinical applications.

That the laboratory worker, the syphilographer, the clinician, and the pathologist may have at hand in one volume a clear, comprehensive, and yet succinct survey of the methods utilized in the application of the complement-fixation test in the field of serum diagnosis is the purpose of this book. That this purpose is excellently well fulfilled the author's reputation would lead one to expect and the book itself fulfills all expectations.

Complement-fixation tests, in the minds of many, suggest only their application to syphilis. That they have, besides this, a wide and varied use is less appreciated and that these applications are here detailed and discussed greatly adds to the usefulness of this volume.

The book is divided into four main sections.

In Part I (seven chapters; 87 pages) are discussed the principles of serum hemolysis and complement fixation; in Part II (22 chapters; 248 pages) the principles of complement-fixation technic are detailed; and in Part III (9 chapters; 80 pages) the minutia of the technic of complement-fixation methods are fully covered.

The relation of Dr. Kolmer to the development of complement-fixation technic assures the reader of a complete, clear, and authoritative presentation.

One is at loss whether to admire more the extent of the author's own investigations; the thoroughness with which every phase of the subject has been studied; the careful analysis of the literature; or the way in which all these have been correlated and combined into a comprehensive whole.

These sections should be read by every worker who takes upon himself the performance of these tests for therein every possible eventuality, every source of error, every puzzling phenomenon is noted and discussed, to the advantage, not only of the laboratory worker who

*Serum Diagnosis By Complement Fixation. With Special Reference To Syphilis. By John A. Kolmer, M.D., Professor of Pathology, Graduate School of Medicine, University of Pennsylvania. Cloth. Pp. 583. 65 illustrations. Lea and Febiger, Philadelphia.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

performs the test, but the clinician who utilizes its results, and who surely should be expected to understand the principles upon which it is based and the factors which may influence its results.

In Part IV (15 chapters; 125 pages) are detailed the methods of obtaining various specimens and a most comprehensive yet succinct discussion of the clinical application of complement-fixation tests.

This book can be read with profit by all who are at all connected with the study of disease and its congeners. It will, however, be a difficult book to borrow as it is apt to be greatly in use.

For the laboratory worker it furnishes a wealth of information concerning all the technical details involved in the conduct of complement-fixation tests, not only in the study of syphilis, but in the study of varied diseases, and the identification of blood stains, meat and milk adulterations and so on; details of every phase from the preparation of the reagents to the conduct of the test.

To the syphilographer it brings under one cover a comprehensive survey of all that is now known concerning the serologic study of syphilis.

To the clinician it furnishes a mine of information of assistance in the understanding and interpretation of the serologic manifestations of syphilis.

In the last analysis it suffices, perhaps, to say that the present volume is worthy to take its place beside the author's previous books, and there are few indeed who are unfamiliar with their worth.

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*Laboratory Diagnosis and Experimental Methods in Tuberculosis**

THE purpose of this book is to describe the more important methods of laboratory diagnosis, to consider the more significant experimental procedures in the study of tuberculosis, and to present such considerations as concisely as possible.

The volume is divided into five principal parts. In Part One the general considerations of the body fluids and excreta in tuberculosis are discussed. Part Two is devoted to the bacteriologic diagnosis of tuberculosis; Part Three to the diagnostic use of tuberculin; Part Four to serologic diagnosis; and Part Five to methods used in the study of experimental tuberculosis.

The author's aim, as stated in the preface, has been "to write a book which would be useful to practicing physicians, to public health officials, and laboratory workers in general, and to medical students."

In this he has been successful. The volume is concise but relatively comprehensive. While, of necessity, many methods of value have not been included, those which are discussed are mainly of demonstrated worth.

The book can be read with profit and is a useful addition to the laboratory library and to the physician's armamentarium. Its study by the physician will undoubtedly decrease the too prevalent "inferiority complex" developed in the presence of the necessity for the diagnosis of tuberculosis in the doubtful case.

Acute Infectious Diseases†

THIS second edition of this well-known work has been so extensively revised that, in many respects, it is almost a new work.

The reputation of the authors is a sufficient guarantee of the comprehensive and authoritative character of the text.

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*Laboratory Diagnosis and Experimental Methods In Tuberculosis. By H. S. Willis, Johns Hopkins Hospital, with a chapter on Tuberculo-Complement Fixation by J. S. Woolley, Loomis Sanitarium, and an introduction by Allen K. Krause. Cloth. Pp. 330. 25 illustrations. C. C. Thomas, Springfield, Ill.

†Acute Infectious Diseases. By J. F. Schamburg, Professor of Dermatology and Syphilology, Graduate School, University of Pennsylvania, and J. A. Kolmer, Professor of Bacteriology and Pathology, Graduate School, University of Pennsylvania. Second Edition. Thoroughly Revised. Cloth, 161 engravings, 27 full-page plates. Lea and Febiger, Philadelphia.

As stated in the preface: "It is a sad commentary upon the intelligence and judicial mindedness of certain elements in the community that it is still necessary to stress the evidence in favor of vaccination."

The present unenviable position of the United States in the World list of smallpox incidence is sufficient evidence that the lessons of the past are not being remembered. It is exceedingly timely, therefore, that a comprehensive and acurate résumé of the vaccination question is available and even the physician will find much of interest in this section of the book.

In the section concerned with diphtheria prevention, a subject now very much to the fore in the public mind, will be found a very excellent discussion of toxin-antitoxin and toxoid immunization.

The authors believe that toxoid immunization has a promising future if present observations are supported by future experience.

The entire volume bears the stamp, not only of the extensive experience of the authors, but of a careful and comprehensive review of the literature. It may be accepted as presenting in a most excellent manner the last word to date of modern medicine on the subject of acute infections. It is a book which the physician can ill do without.

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*Serodiagnosis of Syphilis**

IN THIS small paper-covered volume is presented in succinct fashion the methods used by the author for the conduct of complement fixation and flocculation reactions in the serologic study of syphilis.

Preventive Medicine†

THE present edition of this useful book has been comprehensively revised to include the advances in this important field and may be recommended as authoritative and reliable.

A Laboratory Manual of Physiological Chemistry‡

A TEACHING manual intended for use in medical, dental, or veterinary schools and eminently suitable for the purpose. The book is interleaved for the convenience of the student.

Recent Advances in Bacteriology§

IT WOULD be difficult to select from those concerned with the study of disease any to whom this book would not be useful; the student, the public health worker, the physician, the pathologist, all will find it of interest and value.

The author in his preface says: "The endeavor has been made to take a broad view of many subjects, and, in keeping the balance between extreme technicality and what is already common knowledge, to present a readable exposition of recent work which the general medical reader, not himself especially versed in bacteriology, may appreciate. At

*Precis de Technique du Serodiagnostic de la Syphilis. By R. Demanche. Paper. 123 pages. Gaston Doin et Cie, Paris.

†Preventive Medicine. By M. F. Boyd, M.D., Member of Field Staff, International Health Division of the Rockefeller Foundation. Cloth. 475 pages. Third edition, revised. W. B. Saunders Co., Philadelphia, Pa.

‡A Laboratory Manual of Physiological Chemistry. By D. Wright Wilson, Professor of Physiological Chemistry, Univ. or Penna. Cloth. 272 pages. Williams and Wilkins Co., Baltimore, Md.

§Recent Advances in Bacteriology and the Study of Infections. By J. H. Dible, Professor of Bacteriology and Pathology in the Welsh National Medical School. Pp. 363. 22 illustrations. P. Blakiston's Son & Co., Philadelphia.

the same time the author seeks to indicate to those who do possess some knowledge of bacteriology and the infections, what is being done in spheres outside their own."

The scope of the volume is a sufficient evidence that these aims have been very well accomplished.

There are chapters on the streptococcus problem, bacterial variation, the bacteriophage, Calmette and B. C. G., ultra-microscopic viruses, diseases associated with rickettsia bodies, measles, recent work upon pneumococci, upon spirochetal infections, on diphtheriae and anerobes, and local immunity.

All these are thoroughly and carefully renewed and in an interesting and readable manner. The author shows not only an intelligent mastery of his subject but demonstrates an ability to make it clear to others and to do this in an interesting way.

He has very wisely restricted the bibliography to the papers referred to in the text which are, also very wisely, those referring to more recent work.

This volume can be very highly recommended.

The format and typography are commendable.

A *Handbook of Clinical Chemical Pathology**

THE clinician desiring to utilize to the fullest advantage the resources of modern medicine soon realizes the difficulty of preparing textbooks or systems which are in all respects consistently in step with rapidly changing subjects such as the application of chemistry to the study of disease.

There is a place, therefore, for a succinct yet accurate statement as to the best utilization of such methods and their clinical interpretation.

This is the purpose of this small volume, the first the reviewer has seen from abroad although similar American texts have appeared before.

This little book should be decidedly useful to the practicing physician and lead to a better utilization of the chemical aids now available to the management of disease.

Thrombo-Angiitis Obliterans†

THIS very timely little monograph from the Mayo Clinic is based upon a careful and systematic study of more than 300 cases, fifty amputated specimens being studied pathologically.

This peculiar disease, peculiar in its age and sex frequency and racial predilection, has been the subject of increasing interest in recent years and the present volume presents, not only a summation of the important recent contributions concerning it, but a very excellent intensive study demonstrating many facts of practical interest and suggesting inferences of marked importance.

During the years 1922 to 1927 inclusive the ratio of patients with this disease to all males registering at the Mayo Clinic has been 1:400.

The authors do not regard tobacco as a primary etiologic agent but believe it may be a contributing factor. It is probable, they say, that the racial element depends largely on the clientele and geographic distribution of the physicians who are interested in this disease and that the predominance of the disease in males is related to differences in occupation.

The possible infectious nature of the disease has been the subject of much study. The authors hold that, while proof is lacking, there is much evidence in favor of an infectious or bacterial toxic agent as the etiologic factor, although, of course, there are many con-

*A Handbook of Clinical Chemical Pathology. By F. S. Fowweather, Lecturer in Chemical Pathology, University of Leeds. Cloth. Pp. 216. 18 illustrations. P. Blakiston's Son & Co., Philadelphia.

†Thrombo-Angiitis Obliterans, Clinical, Physiologic and Pathologic Studies. By George E. Brown, M.D. and Edgar V. Allen, M.D., Division of Medicine, Mayo Clinic, Collaborating in Pathology with Howard R. Mahorner, M.D., Fellow in Surgery, The Mayo Foundation. 219 pages. 62 illustrations. Cloth. W. B. Saunders Co., Philadelphia and London, 1928.

tributing elements, such as age, exposure, occupation, etc. They believe that an intensive bacteriologic study is in order.

The results of their pathologic study suggest that thrombo-angiitis obliterans is fundamentally a chronic inflammatory condition of the vessels accompanied by proliferation of the intima and resulting in thrombosis with organization and canalization of the clot, fibrosis of the intima, and an attempt on the part of the vasa vasorum and other collateral channels to establish a collateral circulation. The nerves are involved by virtue of their relationship to the vessels and by ischemia in the distal portions.

In the chapters on the clinical course, clinical types, analysis of symptoms, diagnosis and treatment there is a wealth of practical material very clearly discussed.

The studies of recent years have very materially modified the previous dictum of "high and early amputation" and demonstrated that:

1. In selected cases low amputation is successful.
2. In many cases severe pain is relieved by medical measures.
3. In many cases without trophic changes gangrene can be prevented.
4. Small trophic ulcers can be healed frequently.
5. Lumbar ganglionectomy is occasionally of value.
6. Many patients go through the course of the disease without trophic changes or gangrene and eventually reach a stage of adequate compensation.

Although, therefore, a high degree of individualization is necessary, the authors believe that the type of treatment can be determined by the clinical type of the case when first seen.

The chapters on treatment, prophylaxis, and prognosis well repay careful reading.

A special chapter is given to special methods of investigation.

The typography of the book is excellent.

This volume is a very valuable contribution to the subject and should be in the hands of every physician, most especially, those particularly interested in this condition.

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*Morphologic Variation and the Rate of Growth of Bacteria**

THIS is Volume I of a projected series of Microbiology Monographs designed to cover the field of general, agricultural, and industrial microbiology.

Microbiology is assumming such broad outlines that the only way in which the various phases of this comprehensive subject can ever be covered is by monographic presentation. This volume, therefore, will be welcomed as a thorough, authoritative, clearly written presentation of a rather complicated phase of a far from simple subject.

Fortunately for the average reader Dr. Henrici admits in his preface that he is no mathematician. "The expert in biometrics," he says, "will therefore find here no such carefully fitted curves and elaborate formulae as delight his heart, but the ordinary person may follow my argument with only a very elementary knowledge of statistics." The subject being one in which the "ordinary person" interested in microbiology must be cognizant of, this is, indeed, a consummation devoutly to be wished for!

Recent developments in bacteriology have given a death blow to the old monomorphistic concept which so long dominated the field. The evidence presented by Dr. Henrici makes plain to the average reader just what has been done and what is being done in the study of morphologic bacterial variation, of senescent forms, and of cytomorphosis.

There is a chapter on technic for those desirous of pursuing similar studies, an appendix of tables, and a complete bibliography of the titles referred to in the text.

The author is to be congratulated upon a valuable contribution and the publishers upon an excellent example of craftsmanship.

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*Morphologic Variation and the Rate of Growth of Bacteria. By A. T. Henrici, M.D., Professor of Bacteriology, University of Minnesota. Cloth. Pp. 194. 36 figures. 27 tables. C. C. Thomas, Baltimore, Md.

*The Fuel of Life**

IN THIS volume are presented four lectures delivered under the Louis Clark Vanuxem Foundation of Princeton University.

Dr. MacLeod here presents in a clear and masterly manner what is known at present of the preparation of food materials for combustion in the animal organism, especially as concerns the question as to whether fats as well as proteins form carbohydrate before being used as fuel.

Much of the data presented is based, very naturally, on investigations conducted under Dr. MacLeod's supervision.

His own hypothesis, which these lectures in large measure support, is centered upon the work of Hill and Meyerhof, that the energy of muscular contraction is based largely, if not entirely upon the combustion of carbohydrate. According to his theory neither fat nor protein is burned by the muscles until it has been converted into carbohydrate or some related substance, mainly by the liver.

To present the evidence accumulated in support of this theory is the purpose of the lectures.

The volume is of great interest to physicians, physiologists and all directly or indirectly concerned with human metabolism.

It can be read not only with profit but with interest. A bibliography and a very complete index are included.

Organic Laboratory Methods†

IT IS difficult to conceive a chemical laboratory in which this book will not be received with enthusiasm.

The analytical chemist will find in it the solution of many difficulties. As a reference source it should be invaluable, especially as it appears to be the only book of its kind.

It may be purchased with entire confidence that it will be useful.

Urinary Analysis and Diagnosis‡

THE outstanding feature of this book is the exhaustive and detailed study of the formed elements in urine sediments. The author is firmly convinced, and makes an excellent argument for his case, that much valuable information is lost by neglect of careful study of the epithelium in urine.

The chemical examination of urine is handled in orthodox style though no mention is made of some of the newer and valuable methods such as Exton's method for quantitative albumin determination, for example. That portion of the book concerned with microscopic examination is the best and, if only for this portion, the book is a good reference work.

Neoplastic Diseases§

DR. EWING's masterly treatise on tumors needs no introduction to the medical world for it is doubtful if there is a library of any worth in which it cannot be found showing the honorable scars of frequent use.

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*The Fuel of Life. By J. J. R. MacLeod, Professor of Physiology, University of Toronto. 147 pages. Cloth. Princeton University Press, Princeton.

†Organic Laboratory Methods. Professor Lassar-Cohn. Authorized translation by R. E. Oesper, Ph.D. Assistant Professor of Analytical Chemistry, Univ. of Cincinnati. Cloth. 469 pages. 186 figures. Williams and Wilkins Co., Baltimore, Md.

‡Urinary Analysis and Diagnosis. By L. Heitzmann, M.D. Fifth Revised Edition. 131 illustrations. 366 pages. W. Wood & Co., New York.

§Neoplastic Diseases. By James Ewing, M.D., Sc.D., Professor of Pathology at Cornell University Medical College, New York City. Third Edition. Revised and Enlarged. Octavo of 1127 pages with 546 illustrations. Cloth. 1928, Philadelphia and London: W. B. Saunders Co.

This, the third edition, has been thoroughly and extensively revised, every chapter showing numerous additions.

The chapter on Bones is practically new, having been entirely rewritten. The subjects of mammary cancer and brain tumors are also in large part rewritten.

None who have read the previous editions will want to be without the present edition of this masterly work.

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*The Kahn Test**

THIS volume presents with a wealth of detail the various minutia applying to the technic of the Kahn test and is intended primarily for use in the laboratory.

It will be found of great value to all who are interested in this method and its study will without doubt conduce to its performance in a uniform manner.

This reviewer has always regarded the Kahn test as a valuable *adjunct* to the serologic study of syphilis and has always opposed the clamor for its *exclusive* use especially when urged on the ground of simplicity of technic. He is glad, therefore, to welcome this book which so clearly sets forth the inherent complexities of the reaction and the necessity, therefore, for its performance by trained workers.

An extended bibliography is appended of papers concerning the Kahn test. None of these, however, are discussed in the text.

This book should be in the hands of every worker who essays the Kahn test.

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*The Kahn Test: A Practical Guide. By R. L. Kahn, Sc. D Cloth, 201 pages. 35 tables. 7 plates. Williams and Wilkins Co., Baltimore.

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EDITORIALS

Our Carbon Monoxide Environment

ALTHOUGH present-day conveniences, such as the gas range, the central heating plant, and the automobile have increased the possibility of toxic carbon monoxide exposure, this poison like most other things of which we today take cognizance was a feature of ancient antiquity. Shumway tells us that Aristotle, three hundred years before Christ, wrote that "men suffer from heaviness of the head and often die from coal gas." Valerius Maximus wrote that "Hannibal forced the inhabitants of Nuceria, each with two suits to leave the city, whereupon he prepared baths and caused the people to be suffocated in them by steam and smoke." Thus it was that two hundred years before Christ water gas was an accepted method of execution.

From this we can readily understand that no definite date can be fixed as the time at which carbon monoxide came to be seriously considered as a

cause of disease. Attention was however focused on it in the last decade of the last century by the work of Haldane who was called upon to make a study of the lethal gases in the London tubes in which the trains at that time were drawn by coal-burning locomotives. Haldane concluded after an exhaustive study that the concentration of carbon monoxide in the tunnels should not be allowed to exceed one part per ten thousand.

In recent years Henderson and Haggard have made comparable studies in connection with the vehicular tunnel beneath the Hudson River. They concluded that the maximal allowable carbon monoxide concentration might be four parts per ten thousand. In the course of their investigation they formulated a general rule of toxicity which is easily applicable to all such studies and which at the same time reconciles their allowable content with the lower content recommended by Haldane in England.

They believe that when the duration of exposure expressed in hours multiplied by the carbon monoxide concentration expressed in parts per ten thousand gives a figure not over three no symptoms of intoxication will result. When the exposure time multiplied by the concentration gives a figure of six, symptoms become barely recognizable. When exposure times concentration equals nine, symptoms such as palpitation, headache, vertigo, and sometimes nausea ensue; and when the product equals fifteen the result may be serious.

In the London tube the train crews were to be exposed for several hours at a time. Six hours' exposure, according to Henderson and Haggard's table, to a concentration of one part in ten thousand would give barely recognizable symptoms and it is therefore easy to understand why the concentration must be kept below this figure. In the Holland tube beneath the Hudson, four parts per ten thousand are allowed on the calculation that a maximum of one hour might be required to make the traverse of the tunnel. The investigators further stipulated that the guards at their posts in the tunnel must have specially purified air of a lower concentration.

When we observe that various investigators have agreed that the air in congested streets in our large cities at the peaks of automobile traffic often equals one part per ten thousand and not infrequently even two parts, we must realize that there is actually a carbon monoxide problem. In congested down-town districts we are sometimes actually breathing air that is more vitiated with carbon monoxide than is allowed in the underground tunnels of London.

As one would expect, those exposed for prolonged periods in this vitiated atmosphere actually do experience symptoms therefrom. A careful study has been made by Wilson, Gates, Owen and Dawson of the degree of carbon monoxide absorption among Philadelphia traffic officers. Where the exposure was for relatively long intervals in the congested traffic district they found as high as 30 per cent saturation of the hemoglobin in the blood with carbon monoxide, and these officers experienced definite symptoms, such as rapid pulse and respiration, palpitation and after slight exercise, dizziness and amblyopia. Headache and nausea were not infrequent manifestations.

Connolly in investigating the carbon monoxide hazard in city streets found that on a double-decker drive the vitiation of the air on the lower

deck where breezes could not carry away the gases satisfactorily, remained high enough to be a source of danger to any who might be exposed for a sufficiently long interval. And immediately adjoining this lower deck were windows of tenements in which the occupants spent a good portion of the day. These people certainly are living in a carbon monoxide environment.

The air vitiation in garages often becomes too great for health, and there are records of many cases of poisoning therefrom. Bloomfield and Isbell in a study of a relatively large number of garages through the country found an average carbon monoxide concentration of from 1 to 1.6 parts per ten thousand. At times the content was as high as 8.9 parts per ten thousand. The maximum average concentration observed for a period of one hour was 4.3 parts per ten thousand.

The New York division of Industrial Hygiene made, in 1923, a survey of 157 garages, service stations and repair shops. In the workers in these places 69.5 per cent showed some carbon monoxide in the blood; 77.5 per cent of the garages and other places showed appreciable amounts of carbon monoxide in the air.

A man's blood may become over 20 per cent saturated with monoxide while he is working for one hour in a garage containing four parts per cent thousand concentration.

The exhaust gas from automobiles varies in its carbon monoxide content depending upon carburetor adjustment but it usually contains from four to six or occasionally 10 per cent carbon monoxide. This is rapidly diluted so that a man standing behind a car which is not in motion but in which the engine is going will be in an atmosphere of about four parts per ten thousand. Thus if you are held up in traffic behind an idling engine you are in a decidedly vitiated atmosphere.

We should further bear in mind that physical exertion increases the oxygen demand and therefore will result in symptoms of poisoning earlier than where the victim is at rest. Henderson and Haggard's tables of exposure times concentration mentioned above are for individuals at rest and the effect is doubled or even trebled by exercise. In other words for a person at rest an exposure of three hours in a concentration of three parts per ten thousand will give rise to symptoms, while with an individual at work an exposure of but one hour to the same concentration will theoretically give rise to as severe symptoms.

Acute carbon monoxide poisoning, more often than not, fatal, is well known and incidences thereof are described frequently both in the medical and lay presses. Where death has followed illuminating gas poisoning or the running of a motor in a closed garage the situation is usually obvious at once. The rapidity with which such poisoning may occur is illustrated in the work of Henderson who says that in a closed garage ten feet broad, ten feet high and twenty feet long containing two thousand cubic feet capacity, a car delivering one cubic foot of carbon monoxide per minute will vitiate the atmosphere in one minute, and in five minutes the concentration will reach twenty parts per ten thousand. Beck states that in such a garage the danger point is reached within three minutes and asphyxia may occur in

five minutes. Illuminating gas which often contains as high as 30 per cent carbon monoxide exerts its deadly effect as rapidly. In an atmosphere containing 1 per cent carbon monoxide the victim's hemoglobin will become 50 per cent saturated with carbon monoxide within fifteen minutes, and in twenty minutes coma will ensue.

Carbon monoxide has from 220 to 250 times the affinity for hemoglobin that oxygen possesses. Symptoms appear when the hemoglobin is from 20 to 30 per cent saturated. When the saturation reaches 50 to 55 per cent, walking is difficult, and the victim becomes unconscious at 60 per cent saturation. Dogs die in a carbon monoxide hemoglobin saturation of 84 per cent. In illuminating gas, they die when the carbon monoxide saturation is 72 per cent. Therefore there is something in illuminating gas besides the pure carbon monoxide that also exerts a toxic action.

But we have said that the acute fatal case is obvious. It is the chronic case, the case of the individual exposed to low concentrations over long periods that is worthy of special attention, for there can be no doubt that hundreds of such cases are overlooked daily in the routine work of the physician. One need but be on the alert for such cases to find them. The stenographer working in a closed room heated by a portable gas heater, or at an open window in an air shaft into which a neighboring chimney is belching smoke, or on the ground floor, down town where motor traffic is heavy may complain to you of headache and you may inquire into the efficiency of the office illumination, or you may send her to have glasses fitted, or you may search for focal infection and chronic toxemic absorption from the colon, but the cause of the headache may be none other than carbon monoxide poisoning in its chronic form.

The textbooks dwell at no great length upon this disease nor is much time allotted to it during undergraduate instruction. Were it otherwise there would probably be many more descriptions of carbon monoxide poisoning in the literature.

The pathology is simple. It has apparently been demonstrated that carbon monoxide is not in and of itself a direct tissue poison. Its only activity is a combining with the hemoglobin to the exclusion of oxygen, and the resulting pathologic changes are those of anoxemia. Henderson was able to grow embryonic chicken brains in chick plasma in a concentration of 21 per cent oxygen and 79 per cent carbon monoxide. Hemoglobin does not enter into this procedure and the available oxygen is normal. The 79 per cent carbon monoxide behaved as an inert gas just as nitrogen does in our own respiratory air. If an animal breathes oxygen under two atmospheres pressure, thus rendering the oxygen content of the blood independent of hemoglobin, the addition of carbon monoxide in any amount produces no symptoms.

The pathologic physiology being an anoxemia we may readily understand how practically any tissue of the body may undergo pathologic change. The nervous system being most susceptible to anoxemia usually shows the more extensive changes but in fatal cases, especially, other organs and tissues may suffer. Briefly the changes in the nervous system consist in perivascular

hemorrhages followed by softening. Any part of the nervous system may be so affected. The lenticular nucleus appears to be especially susceptible.

Alpers describes a nonfatal case of poisoning in which the nervous manifestations indicated damage to the lenticular nucleus, the pyramidal tract, the bulbar nuclei and the peripheral nerves in the form of a peripheral neuritis. R. M. Stewart describes a fatal case with bilateral softening of the corpus striatum, softening and hemorrhages of the deeper layers of the cortex and cerebellum, but with the chief damage in the corpus striatum. Ruge describes twelve cases of carbon monoxide poisoning with softening and changes in the vessels of the brain, especially in the lenticular nucleus. He observes in moderately severe experimental poisoning, fatty degeneration in the ganglion cells within twenty-four hours of exposure, with small hemorrhages in the perivascular spaces. After two days, softening of the lenticular nucleus appears but this is not well marked until about the fourth or fifth day.

There may be scattered small hemorrhages and intense hyperemia of all body organs. There may be a great variety of eye lesions such as scotomas, color blindness, extraocular palsy, engorgement of the retinal vessels, impairment of the pupillary reflexes, irregularity of the pupils, nystagmus, edema of the optic discs followed by secondary optic atrophy and blindness, retinal hemorrhages or even complete ophthalmoplegia. Blindness is rare but when it does occur it is usually due either to an optic neuritis or to central changes, presumably softening in the region of the visual fields.

It has been said that chronic carbon monoxide poisoning does not occur unless the fixed carbon monoxide in the blood amounts to at least 5 per cent of the hemoglobin saturation. Fortunately methods are available by which the carbon monoxide saturation may be determined down to 1 per cent.

Early symptoms of monoxide poisoning are "toxic." They consist of yawning, sleepiness, weariness, constriction across the forehead, frontal headache, at first intermittent but later continuous and more severe. This headache is later replaced by the typical monoxide headache located at the base of the skull, in the occipital region, extremely severe, causing the sufferer to hold his head back as far as possible. This typical headache has been attributed to increased intracranial pressure.

Then come dizziness and nausea, rapid heart and respiration, cardiac irregularity and finally stupor. Vomiting sometimes occurs. After recovery the headache, weakness and often loss of memory persist for several days.

The symptoms of chronic monoxide poisoning are rather more insidious. Shumway describes them as headache, palpitation, anorexia, vertigo, anemia, eyestrain, general fatigue, lack of concentration, neuralgic pains, congested nasal and ocular mucous membrane, dry throat, irritation and watering of the eyes, lack of decision, restlessness, irritability, insomnia, and sometimes visual and auditory hallucinations.

These are the usual symptoms of many chronic intoxications and this is the more reason why the physician should bear in mind the present-day frequent sources of chronic carbon monoxide poisoning. As Lewin of Berlin states, there is no poison which is known to give so wide a variety of patho-

logic changes as carbon monoxide. The same could almost be said for its symptoms.

Beck has made an especially comprehensive study of the symptomatology of chronic monoxide poisoning and its manifestations are surely protean. At first there is a compensatory polycythemia with an increase both in the red count and in the hemoglobin concentration. Later this gives place to an anemia which may often closely resemble pernicious anemia. Indeed it may have many of the characteristics of pernicious anemia including the atrophic glossitis, the gastric achylia, asthenia, high color index and even the paresthesias which in pernicious anemia are customarily due to a posterolateral sclerosis. Beck further remarks that in chronic monoxide poisoning the glossitis may be present even without the anemia and its observance should remind one of the possibility of this form of poisoning. According to this author abdominal symptoms are not uncommon and are due to increased tonus of the smooth muscle system. The symptoms may even be so acute and severe as to suggest perforation of a gastric ulcer or renal colic. He describes cases clinically resembling hyperthyroidism, with exophthalmos, sweating, etc., due to the action of the poison on the autonomic nervous system but in which the pulse and basal metabolic rate remain normal.

Usually psychasthenic and neurasthenic symptoms are prominent. Beck emphasizes the diagnostic significance of a symptomatology usually attributed to anemia, in an individual whose blood examination actually shows a polycythemia.

The earliest and most constant eyeground changes consist in congestion in the retinal vessels and hyperemia of the optic discs. These are worth looking for in a suspected case.

We should bear in mind that children are much more susceptible to carbon monoxide poisoning than adults and that a child may even be fatally poisoned in a room in which an adult companion experiences little or no symptoms. Abt reports the case of a child who although not fatally poisoned was blinded permanently from exposure to illuminating gas while playing in the same room with his mother who at no time was conscious of toxic symptoms.

This phase of the situation is especially important with infants who are unable to describe their symptoms or to ask for help. Stevens describes the evidences of chronic monoxide poisoning, as failure to gain in weight, attacks of vomiting, restlessness, constant crying, anorexia and night terrors. It is only in the advanced cases that we observe the telltale pink flush of the skin, the slow pulse and the subnormal temperature. Here again the symptoms are symptoms that might be attributable to any of many causes and this is so much the more reason for bearing the possibility of monoxide poisoning in mind. Proof that the symptoms are due to this cause may be obtained by examination of the blood or by changing the environment, after which the infant commences to flourish.

Fortunately for the acute case the poison is eliminated relatively rapidly, the major portion being removed in from three to five hours, but minute amounts may persist in the blood for two weeks or longer, and where this is

cumulative and the exposure to small amounts continues, chronic poisoning may supervene.

Fortunately the means are available for recognizing carbon monoxide either in the atmosphere or in the blood. Air samples may be obtained over varying intervals by drawing air into properly arranged tanks, or instantaneous specimens may be obtained by the so-called *grab* method. A mason jar filled with water suddenly inverted allows the replacement of the water with air from the locality to be tested. The cap is then quickly applied. Another grab method consists in inflating the bladder of a basket ball with an air pump. In this case the determination must be made relatively soon thereafter to prevent the diffusion of gases through the rubber.

Probably the best method for air analysis in detecting carbon monoxide is the iodine pentoxide method. A quick qualitative method consists in exposing a saturated solution of lime water to the air. Carbon monoxide causes rapid clouding with precipitated calcium carbonate.

For examination of the blood the Van Slyke and Salvesen method is accurate down to 1 per cent. A rapid qualitative method which will establish the presence of 10 per cent or over of monoxide hemoglobin saturation consists in laking three or four drops of blood in five c.c. of distilled water and then adding a drop or two of 5 per cent sodium hydroxide. Normal blood turns a dirty green with this procedure while monoxide blood remains unchanged in color, preserving its original pink, or if more pronounced, cherry red color.

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—*W. T. V.*

The Oral Administration of Typhoid Vaccine

THE oral administration of bacterial vaccines was proposed as long ago as 1904 and ever since has been the subject of sporadic investigation with rather indefinite results.

Following some rather promising results in animals Besredka[1] in 1922 during an epidemic immunized 268 students by the oral administration of typhoid vaccine, a similar group of 253 being vaccinated subcutaneously. In the first group five new cases developed after eleven days while ten new cases developed after twenty days in the second group.

Results similar in character are reported by others, notably Gauthier[2] who reports 4000 cases.

Opposed to this is the report of Achard and Blach[3] who failed to find either agglutinins or complement fixing bodies after oral vaccination.

Because of the paucity of experimental evidence, the question has again been studied by Haffstadt and Thompson.[4]

A group of 93 university students from nineteen to forty years of age furnished the experimental material, 5 students being vaccinated subcutaneously as controls.

Of the experimental group 55 received 1 c.c. of triple typhoid vaccine in a glass of water one-half hour before breakfast on three consecutive days; and 38 received two capsules each containing 1.5 grains of alcoholic extract of ox bile with the first of the three doses of vaccine.

The first group were tested after five and nine months and the second after five months for the presence of agglutinins, precipitins, and complement-fixing bodies.

Agglutinins were tested for by the macroscopic method, a total of 30,000 agglutination tests being made during the experiment with the results summarized below.

The systemic reaction was much less after oral than subcutaneous vaccination. Of the entire group of 93, 88.5 per cent showed agglutinins for typhoid and a lesser number for the paratyphoids, the agglutinin titer being higher in the bile-prepared group, the bile appearing to hasten slightly agglutinin production. The majority of cases showed a latent period of three to four weeks. The highest agglutinin titer for typhoid was 600, for paratyphoid 100, the maximum titer being reached by the time of the last subcutaneous inoculation and in the fourth and fifth week after oral ingestion of the vaccine. The disappearance of the agglutinins was too varied to warrant any generalization.

From this portion of their study the authors concluded that:

1. Agglutinins can be produced by the oral administration of triple typhoid vaccine.
2. There is no delay in the appearance of agglutinins thus produced.
3. Bile seems to aid agglutinin production.

4. Previous treatment or history of typhoid fever affects only the agglutinin titer of the individual.

5. Immunity may be demonstrated nine months after treatment.

In a continuation of their study Haffstadt and Thompson investigated the incidence and degree of production of precipitins and complement-fixing body production in the group (39) subjected to oral vaccination.

Precipitins appeared in 66.6 per cent and complement-fixing bodies in 60 per cent. The administration of bile was without apparent influence on the production of these immune bodies. The latent period for complement-fixing bodies is four to five weeks, precipitins appearing somewhat earlier. The disappearance of these antibodies is irregular and their duration variable.

There seems to be little relation between the presence of the various antibodies, but few individuals showing all three, and neither precipitins nor complement-fixing bodies being consistently found. There was, however, an apparent relation between the appearance and disappearance of complement-fixing bodies and agglutinins.

A still further study showed that entirely similar immunologic results were obtainable after the oral administration of the vaccine in capsules prepared after the formula following:

A suspension containing four billion typhoid organisms (Rowling's strain), 28 million para A, and 28 million para B, per centimeter was titrated with a matrix of starch until the moisture was absorbed. After adding an alcoholic extract of bile it was dispensed in 00 capsules each containing 0.25 c.c. of the original suspension and 1.5 grains of ox bile. One capsule was given one-half hour before breakfast on three successive mornings.

The experimental evidence thus adduced entirely corroborates the clinical evidence heretofore reported and demonstrates a scientific basis for oral typhoid vaccination.

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